THE PHILIPPINE
JOURNAL OF SCIENCE

VOL. 116; JANUARY – MARCH 1987 NO. 1

STABILITY STUDIES OF ESSENTIAL OILS FROM SOME PHILIPPINE PLANTS I. *ZINGIBER OFFICINALE* ROSC.

MINDA FLOR T. BRANDARES, B. B. DAR JUAN, A. M. VUELBAN
and F.E. ANZALDO
Pharmaceutical Research Program
National Institute of Science and Technology

ABSTRACT

Ginger oil was extracted from the rhizomes of *Zingiber officinale* Rosc. by hydrosteam, steam and water distillation methods. Hydrosteam distillation gave the highest yield.

The effects of temperature, light and length of storage on the rate of oil evaporation and its quality were determined. The oil volatilizes fastest when exposed to sunlight while refrigeration retards its evaporation. A comparison of thin layer chromatograms of oil samples exposed and unexposed to light taken monthly for 7 months showed a decrease in the number of spots, showing deterioration of the quality of the oil.

The degree of volatility of ginger oil obtained from fresh, frozen and air-dried samples were also determined. Of the oil samples, the oil from the air-dried sample gave the lowest rate of evaporation.

INTRODUCTION

Ginger *(Zingiber officinale* Rosc.), a very common plant in the Philippines, is popularly used by the herb "doctors" or "herbolarios" in the country. Listed among their purported uses are their effectivity against beri-beri, centipede bites, cholera, cough and colds, early stage of goiter, influenza, headache, hypertension, muscular pain, rheumatism, sore eyes and ulcer (Quintana, 1979). The essential oil is used in the pharmaceutical industry as an ingredient of lozenges, cough preparations and liniments. In the Pharmaceutical Research Program of the National Institute of Science and Technology, a cough candy using ginger oil with other fruit extracts was formulated which was found effective against some test organisms (Brandares *et al,* 1986). A ginger tincture was prepared by Concepcion and Concha (1975) using the USP process M method.

In the food industry, it is used as an aromatizer and flavorant. The rhizomes of ginger are used as a condiment, one of the most famous flavorants known and the popular native beverages such as "salabat", ginger ale and ginger beer are prepared from it. A proteolytic enzyme extracted from the rhizome is used as meat tenderizer (Reyes and Ruiz, 1976).

Ginger used to be just a backyard crop but recently ginger farming has undergone expansion. Although at present, it is sold only in the fresh market, increased production would require some form of processing to accomodate the excess produce. In Hawaii, a processed ginger product, a paste, was prepared from fresh ginger as a convenient shelf-stable product with its odor characteristics (Baranowski, 1985). While refrigeration may retard losses of odor, color and the major pungent component, there was still considerable degradation of the product stored at 10°C over 24 weeks or 8-week storage at 25°-27°C.

In this study, a steam-distilled volatile oil was extracted from ginger to evaluate not only its use as a spice but also as an ingredient in the formulation of pharmaceutical products with a view on its export potential. Depending upon storage conditions, the loss of volatile oil from dried ginger can be fairly rapid and it has been reported that ground dried ginger can lose up to 50% of its volatile oil during 6 months of storage (Richardson, 1967). The yield of the oil may depend on the collection, the season and the age at which the drug is collected since the amount and sometimes the nature of the active constituents is not constant the whole year (Cantoria, 1979).

A literature survey of studies on essential oils up to 1982 showed that there are not many studies on ginger oil done locally (Anzaldo and Coronel, 1982). Recently, Anzaldo *et al* (1986) elucidated on the chemical components of local ginger oil. Among the Zingiberaceae family, extensive studies on the constituents of the volatile oil from some varieties of *Z. zerumbet* were made (Oliveros and Cantoria, 1976). However, none has been reported on the factors affecting the stability of the extracted oil from ginger rhizomes. We have therefore undertaken studies on the effects of temperature, length of storage and light on the stability of the essential oil from ginger. Through this, storage as well as transportation problems may be overcome and therefore enhance the export feasibility of this product.

MATERIALS AND METHODS

Fresh, frozen and air-dried rhizomes of *Z. officinale* were used in this study. The samples were collected from Lemery, Batangas and Catanauan, Quezon and some were purchased from Paco Market in Manila.

Extraction of the Oil

Different distillation methods were tried to determine the procedure which will give the highest yield of volatile oil. Thus, the oil was extracted from the comminuted rhizomes by hydrosteam, steam and water distillation. The oil obtained was separated from the distillate by means of a Clevenger tube attached to the condenser. Anhydrous sodium sulfate was added to free the oil from small traces of water and the oil was stored in the refrigerator.

beverages such as "salabat", ginger ale and ginger beer are prepared from it. A proteolytic enzyme extracted from the rhizome is used as meat tenderizer (Reyes and Ruiz, 1976).

Ginger used to be just a backyard crop but recently ginger farming has undergone expansion. Although at present, it is sold only in the fresh market, increased production would require some form of processing to accomodate the excess produce. In Hawaii, a processed ginger product, a paste, was prepared from fresh ginger as a convenient shelf-stable product with its odor characteristics (Baranowski, 1985). While refrigeration may retard losses of odor, color and the major pungent component, there was still considerable degradation of the product stored at 10°C over 24 weeks or 8-week storage at 25°-27°C.

In this study, a steam-distilled volatile oil was extracted from ginger to evaluate not only its use as a spice but also as an ingredient in the formulation of pharmaceutical products with a view on its export potential. Depending upon storage conditions, the loss of volatile oil from dried ginger can be fairly rapid and it has been reported that ground dried ginger can lose up to 50% of its volatile oil during 6 months of storage (Richardson, 1967). The yield of the oil may depend on the collection, the season and the age at which the drug is collected since the amount and sometimes the nature of the active constituents is not constant the whole year (Cantoria, 1979).

A literature survey of studies on essential oils up to 1982 showed that there are not many studies on ginger oil done locally (Anzaldo and Coronel, 1982). Recently, Anzaldo *et al* (1986) elucidated on the chemical components of local ginger oil. Among the Zingiberaceae family, extensive studies on the constituents of the volatile oil from some varieties of *Z. zerumbet* were made (Oliveros and Cantoria, 1976). However, none has been reported on the factors affecting the stability of the extracted oil from ginger rhizomes. We have therefore undertaken studies on the effects of temperature, length of storage and light on the stability of the essential oil from ginger. Through this, storage as well as transportation problems may be overcome and therefore enhance the export feasibility of this product.

MATERIALS AND METHODS

Fresh, frozen and air-dried rhizomes of *Z. officinale* were used in this study. The samples were collected from Lemery, Batangas and Catanauan, Quezon and some were purchased from Paco Market in Manila.

Extraction of the Oil

Different distillation methods were tried to determine the procedure which will give the highest yield of volatile oil. Thus, the oil was extracted from the comminuted rhizomes by hydrosteam, steam and water distillation. The oil obtained was separated from the distillate by means of a Clevenger tube attached to the condenser. Anhydrous sodium sulfate was added to free the oil from small traces of water and the oil was stored in the refrigerator.

Physico-chemical analysis of the essential oil

Thin layer chromatography experiments were performed on the essential oils obtained from the rhizomes in various states, namely, fresh, frozen and air-dried and the number of spots determined. The spots were obtained by TLC on silica gel G using ethyl acetate: benzene (1:19) as solvent and iodine as developer. For comparative purposes, any change in the number of spots in the chromatogram served as a gauge of the change in the quality of the oil.

The refractive index and specific gravity of the oil were determined using the Abbe refractometer and pycnometer, respectively (Jenkins *et al,* 1957). The acid number was obtained by titration method and the ester number by saponification with heat (Guenther, 1948).

Solubility tests were performed to determine at which concentration of ethanol the oil was most soluble. Ten drops of oil were dissolved in 5 mL each of 50, 60, 65, 70, 75, 80, 90 and 95% ethyl alcohol.

Stability Studies

Effect of temperature

The degree of volatility of the oil at different environmental temperatures was determined by the following procedure: Three Petri dishes were tared by placing them inside the oven for 1 h at 100°C. After cooling in the desiccator, 0.1 mL of sample was introduced to an empty dish by means of a micropipette and reweighed. Maximum care was observed to prevent the loss of oil in the preparation of the sample. The procedure was repeated on two other dishes. The weighing was done as fast as possible so that the exposure of the 3 could be done simultaneously. One sample was exposed to room temperature, the second sample to direct sunlight and the third placed inside the refrigerator. The temperatures under which the 3 samples were exposed as well as the initial times of exposure were recorded. The samples were allowed to dry and the time complete evaporation was attained and the weight of the samples were noted.

The degree of volatility of the oil was likewise determined at different environmental temperatures within a fixed exposure time. To each of the 3 Petri dishes, about 0.2 mL of ginger oil was introduced and weighed. The first Petri dish was exposed to sunlight, the second at room temperature and the third in the refrigerator. The samples were again weighed after 3 hours exposure to determine the loss of weight.

The effect of temperature on the essential oils obtained from the samples at different states: fresh, frozen and airdried was also studied. About 0.3 g oil extracted from each of the 3 samples was placed in capillary tubes previously dried to constant weight. Three tubes each were then exposed to room temperature (33°C), placed inside the refrigerator (19°C) and oven (40°C), respectively. The samples were weighed after every 15 minutes for two hours.

Effect of light and length of storage

Two 2.5 mL ginger oil from air-dried rhizomes were placed in micro test tubes. One tube was stored in the dark while the other was kept indoor under ordinary light. TLC was performed before storage and every month thereafter for seven months. The number of spots was recorded and the change in color noted.

RESULTS AND DISCUSSION

Of the three methods of ginger oil extraction used, hydrosteam distillation gave the highest yield (0.5 – 1.0%) of volatile oil. Water distillation method was discontinued because of heavy frothing and steam distillation, too, because of the low yield and for economic reasons since it necessitates use of a more complicated set-up.

Ginger oil is a yellow to orange liquid possessing the characteristic aromatic odor, but not the pungent flavor or "bite" of the spice. This piquant nature is due to the oleoresin which has been removed by distillation. The oil is lighter than water with a specific gravity of 0.8803 and a refactive index of 1.4772 at 25°C. It gives average ester and acid numbers of 14.96 and 1.57, respectively.

The thin layer chromatograms of the collected oil from fresh, air-dried and frozen ginger rhizomes showed 10 spots (Fig. 1). These could be ketones, phenols or aldehydes. Anzaldo *et al* (1986) identified these spots to be terpineol, citral, geraniol, limonene, phellandrene, α-pinene, linalool, β-pinene, cineol and caryophyllene by a study of their IR spectra. This result is an improvement on Young's (1969) findings. He reported only 5 components present in the oil as determined by TLC and 4 by gas liquid chromatography.

The oil is soluble in 70 – 95% ethyl alcohol, the degree of solubility increasing with the concentration of the solvent.

The above physico-chemical analyses were performed in order to establish the purity of our sample. The data obtained fall within the range of values reported by Guenther (1952).

Stability Studies

Essential oils are generally unstable and spoilage may be attributed to such general reactions as oxidation, resinification, polymerization, hydrolysis of esters and to interreactions of functional groups. These processes may be influenced by the presence of oxygen, heat, or moisture and catalyzed by exposure to light and possibly by metals (Guenther, 1948).

Ginger oil samples were exposed to different environmental temperatures to determine the time at which the sample would completely evaporate. As shown in Table 1, fastest evaporation was achieved under sunlight. Refrigeration retarded complete volatilization for as long as 67 hours. It was observed that all the 3 samples had evaporation residues, the amount of which depended upon the temperature but which were negligible.

We did not prove the cause nor identity of the residues since this is beyond the scope of our present study. According to Guenther (1948), constant weight cannot conveniently be attained because of the fact that other materials present such as waxes and other high boiling non-volatile materials tend to retain or "fix" some of the lower boiling constituents.

The degree of volatility of the ginger oil at different environmental temperatures were also determined within a fixed exposure time. As expected, the sample exposed to the highest temperature, that is under sunlight, evaporated fastest with a weight loss of 0.0925 g and the refrigerated sample had the lowest evaporation rate of 0.0301 g/3h (Table 2).

We also tried determining the effect of temperature on the oils extracted from samples in various states: fresh, air-dried and frozen to find out whether the effect of temperature on the oil would be the same irrespective of the nature of the sample from which it was obtained. The amount of oil evaporated from the three samples were plotted against the time of exposure at different temperatures.

At room temperature (Fig. 2), the oil from the frozen sample had the highest amount evaporated for the first 15 minutes. After 2 hours of exposure, the total loss of weight of oil from fresh sample was 1.7×10^{-3} g, 1.2×10^{-3} g of oil from air-dried sample and 1.7×10^{-3} g from frozen sample.

The oil from fresh sample gave the highest amount of oil evaporated for the first 15 minutes of oven temperature (40°C) followed by the oil from frozen sample and then from air-dried sample (Fig. 3). The total amount of oil evaporated after two hours was 8.4×10^{-3} g from fresh sample, 7.5×10^{-3} g from fresh sample, 7.5×10^{-3} g from air-dried and 8.6×10^{-3} g from the frozen sample.

Two maximum peaks of evaporation were recorded (Fig. 4) at refrigerator temperature (19°C) after thirty and seventy five minutes, and those were oils from fresh and frozen samples, respectively. Oil from fresh sample evaporated a total of 1.0×10^{-3} g, 8.5×10^{-4} g from air-dried and 1.1×10^{-3} g from frozen samples.

Apparently, oil from air-dried samples gave the lowest rate of evaporation among the three samples. Oils from frozen and fresh samples had almost equal rates of evaporation. These have water-soluble constituents of volatile oil which could have evaporated during the process of air-drying. In most cases, evaporation will affect the more water-soluble constituents of a volatile oil rather than the low-boiling terpenes.

Essential oils distilled from fresh, frozen or air-dried plant parts show variations in physico-chemical properties and chemical composition (Guenther, 1948). Lawrence (1983) reported that of the 81 constituents previously identified, 20 have been found only in the oil of fresh ginger. These components may be preserved in freezing and there are compounds that may remain unchanged after drying.

Comparative studies on the effect of light and length of storage were also undertaken on 2 oil samples by monthly determinations of thin-layer chromatograms. The TLC of the freshly extracted oil samples showed 10 spots (Fig. 1). One sample was stored in a dark place while the other was left in the laboratory exposed to ordinary light. As shown in Figs. 5 and 6, there was a noticeable decrease in the number and an increase in size of the spots of the TLC of both samples; the sample exposed to light deteriorating faster as indicated by loss of more spots. On the 7th month, the unexposed oil had 4 spots left while the exposed one had 3. It was also observed that both exhibited a faded yellow color, the exposed oil having a lighter color. Both, therefore, deteriorated upon long storage under these conditions. These reactions may be due to oxidation or interaction of functional groups catalyzed by exposure to light.

Most essential oils consist of mixtures of hydrocarbons (Lawrence, 1983) like terpenes, sesquiterpenes, etc., oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, phenol ethers, etc.) and a small percentage of viscid or solid non-volatile residues (paraffins, waxes, etc.). The solubility of the oil in dilute alcohol may be attributed to the presence of oxygenated substances which are also the principal odor carriers and with the exception of some aldehydes contribute to greater stability of the oil against oxidizing and resinifying influences. It is due to the unsaturated nature of the terpenes and sesquiterpenes that they oxidize and resinify easily under the influence of air and light or under improper storage conditions (Guenther, 1948).

SUMMARY AND CONCLUSIONS

1. Extraction by hydrosteam distillation of *Z. officinale* Rosc. rhizomes gave the highest yield of oil.

2. Oil extracted from air-dried samples gave the lowest rate of evaporation while oils from frozen and fresh samples had almost equal rates of evaporation.

3. The rate of volatility of oil from air-dried samples was fastest under sunlight and lowest on refrigeration.

4. Quality of oil deteriorated upon storage and deterioration was faster when exposed to light.

ACKNOWLEDGEMENT

Thanks are due to Misses Marilyn Wacan and Marina Recaña and to Messrs. Antonio Balotro and Gabriel Villaluz for their laboratory assistance and to Miss Julia Arandia for the typing of the manuscript.

REFERENCES

1. Anzaldo, F.E., and V.Q. Coronel. 1982. (Abstract) Bibliography on Essential Oils. National Science and Technology Authority, Philippines.
2. Anzaldo, F.E., V.Q. Coronel, J.B. Manalo and C.R. Nuevo. 1986. Chemical components of local ginger oil. *NSTA Tech. J.* **11**(3): 11-19.
3. Baranowski, J.D. 1985. Storage stability of processed ginger paste. *J. Food Sci.* **50**(4):932-933.
4. Brandares, M.F.T., A.M. Vuelban and F.E. Anzaldo. 1986. Cough candy using local plant extracts. *NIST J.* (in press).
5. Cantoria, M. 1979. The physiology of plants yielding volatile oils and alkaloids. *Proc. 28th Ann. Convention,* PhilAAs, Philippines, pp. 51-58.
6. Concepcion, A., and J.A. Concha. 1975. Ginger tincture. *J. Philipp. Pharm. Assoc.* **61**(1-4).
7. Guenther, E. 1948. The Essential Oils. Vol. 1. D. Van Nostrand Co., New York.
8. Guenther, E. 1952. The Essential Oils. Vol. 5. D. Van Nostrand Co., New York.
9. Jenkins, G.L., J.E. Christian and G.P. Hayes. 1957. Quantitative Pharmaceutical Chemistry. McGraw-Hill Book Co., Inc., New York.
10. Lawrence, B.M. 1983. Recent studies on the oil of *Zingiber officinale* Roscoe. Essential Oil Technical Paper Book 4 in IXth International Congress of Essential Oils, Singapore, pp. 69-77.
11. Oliveros, M.B., and M. Cantoria. 1976. A study of the volatile constituents of some species of the Family Zingiberaceae. *J. Philipp. Pharm. Assoc.* **62**:48.
12. Quintana, E.G. 1979. Survey of folk use of potential medicinal crops. In: Proc. 28th Ann. Convention, PhilAAs, Philippines, pp. 31-49.
13. Reyes, S., and E.L. Ruiz. 1976. The efficiency of ginger extract as meat tenderizer. *11th Philipp. Assoc. Food. Tech. Proc.,* **24**: 169-205.
14. Richardson, K.C. 1967. The packaging and storage of dried ginger. *J. Food Sci.* **50**(4): 932.
15. Young, R.G. 1969. Characterization of the essential oils of some Philippine plants. *Philipp. Agriculturist* **53**(2): 100-107.

Table 1. Degree of volatility of essential oil from *Z. officinale* at different environmental temperatures.

	REFRIGERATOR TEMPERATURE (22°C)	ROOM TEMPERATURE (26°C)	UNDER SUNLIGHT (34°C)
Weight of sample before exposure (g)	0.08184	0.07560	0.08060
Weight of sample after exposure (g)	0.05324	0.01510	0.01954
Weight loss after exposure (g)	0.02860	0.06050	0.06106
Time of exposure until complete evaporation (h)	67.08	4.41	2.51

Table 2. Degree of volatility of ginger oil at different environmental temperatures within a fixed exposure time (3 h)

	REFRIGERATOR TEMPERATURE (19°C)	ROOM TEMPERATURE (31°C)	UNDER SUNLIGHT (35°C)
Weight of sample before exposure (g)	0.0785	0.0748	0.0981
Weight after exposure (g)	0.0484	0.0107	0.0256
Weight loss after 3-h exposure (g)	0.0301	0.0641	0.0925

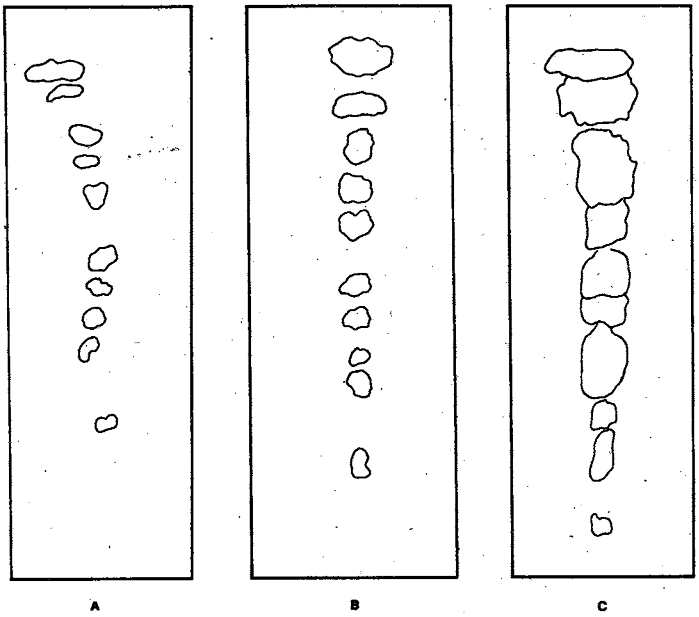

Figure 1. Thin-layer chromatograms of ginger oil from:
A – fresh rhizomes B – air-dried rhizomes C – frozen rhizomes

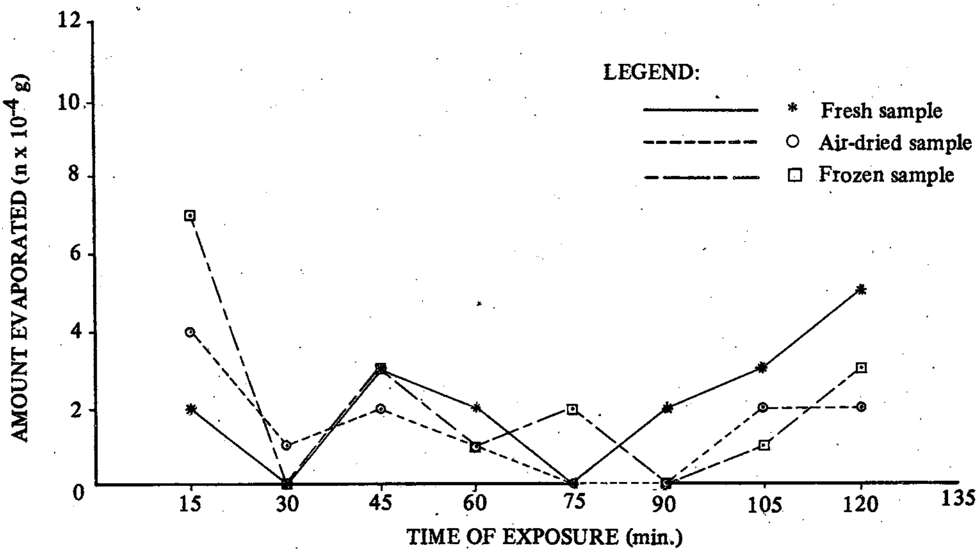

Figure 2. Effect of room temperature (32°C) on evaporation rate of oils from fresh, frozen and air-dried Z. officinale *rhizomes.*

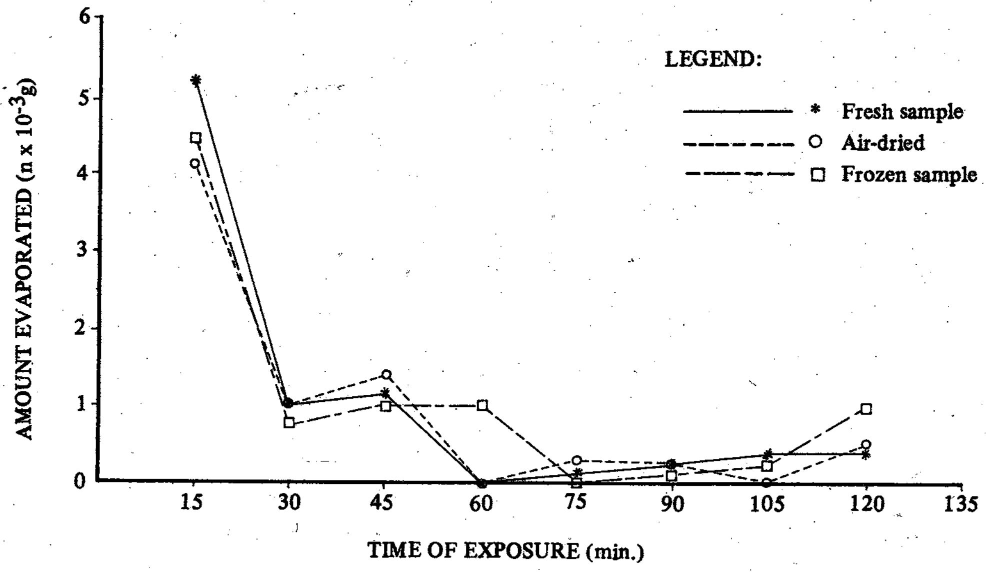

Figure 3. Effect of oven temperature (40°C) on evaporation rate of oils from fresh, frozen and air-dried Z. officinale *rhizomes*

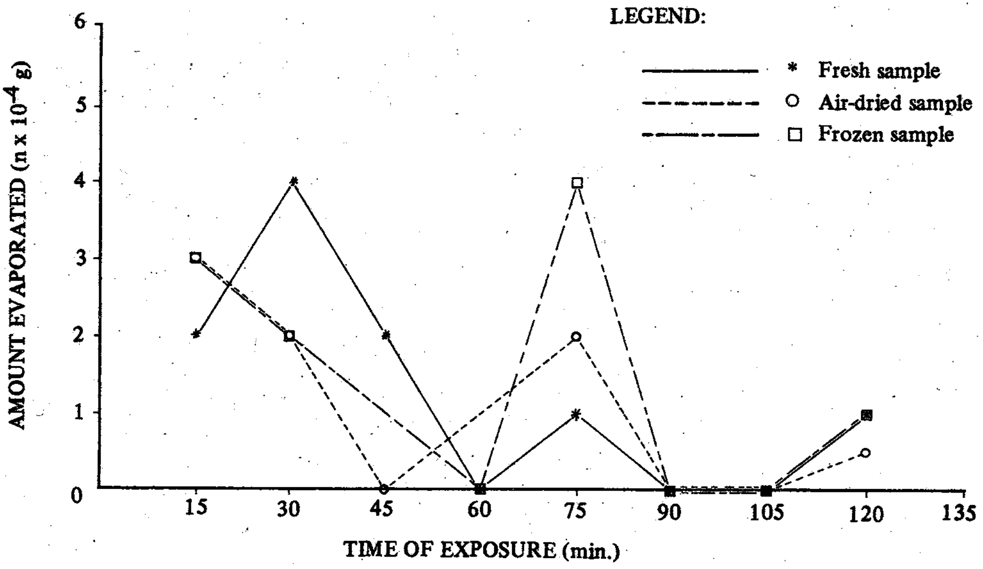

Figure 4. Effect of refrigerator temperature (19°C) on evaporation rate of oils from fresh, frozen and air-dried Z. officinale *rhizomes.*

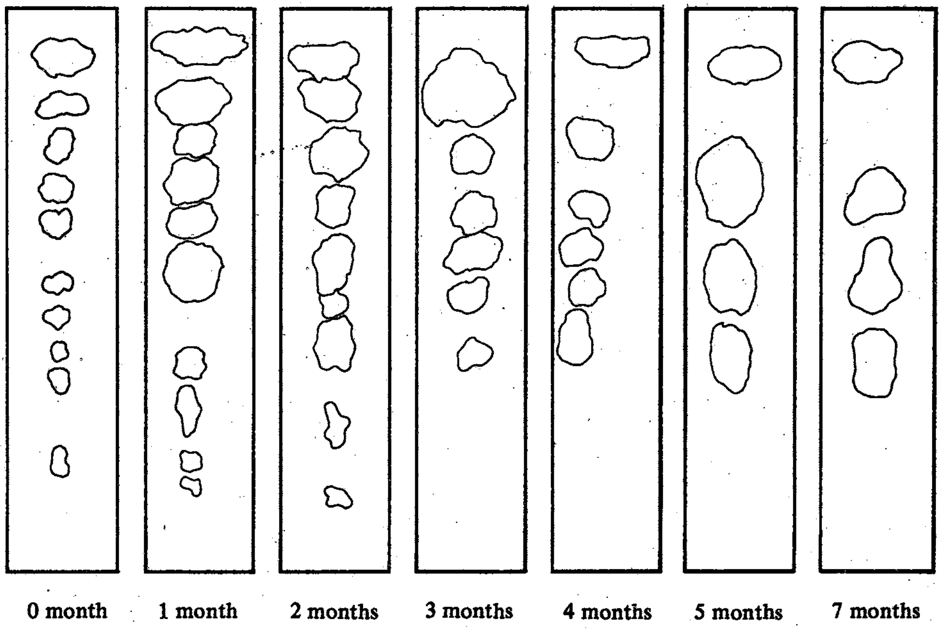

Figure 5. Thin-layer chromatograms of ginger oil from air-dried rhizomes unexposed to light.

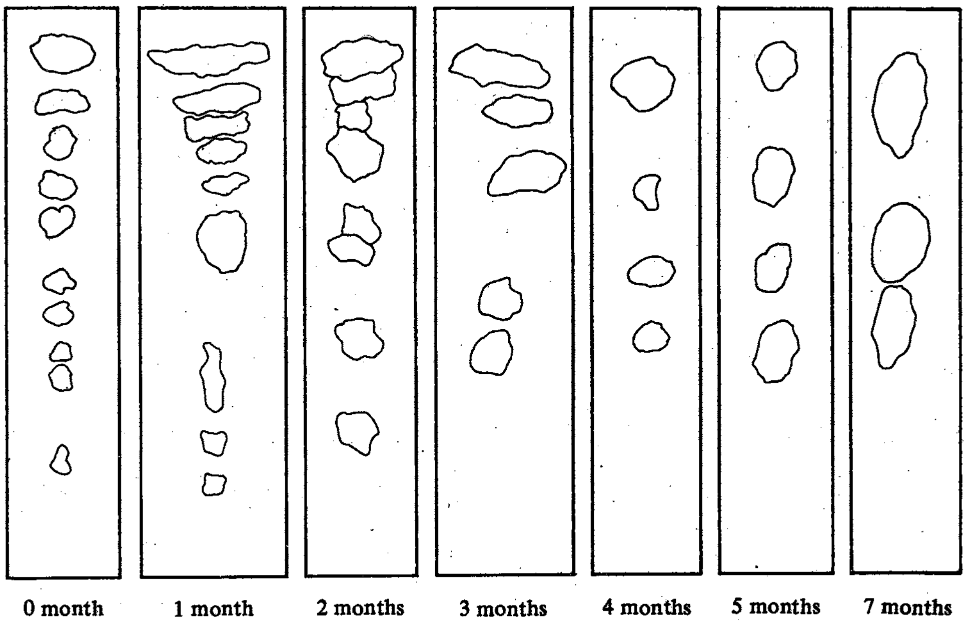

Figure 6. Thin-layer chromatograms of ginger oil from air-dried rhizomes exposed to light.

MUTAGENICITY, CLASTOGENICITY AND ANTIMUTAGENICITY OF MEDICINAL PLANT TABLETS PRODUCED BY THE NSTA PILOT PLANT II. *SAMBONG* TABLETS

C.Y. LIM-SYLIANCO, F.R.B. BLANCO and C.M. LIM
Department of Chemistry, College of Science
University of the Philippines
Diliman, Q.C.

ABSTRACTS

"Sambong" tablets prepared by NSTA Pilot Plant did not show direct DNA damaging capacity. These tablets were not mutagenic before and after metabolic activation and they did not possess chromosome-breaking potential.

However, these tablets reduced the formation of micronucleated polychromatic erythrocytes induced by dimethylnitrosamine, tetracycline and mitomycin C suggesting that "sambong" tablets possess antimutagenic activity.

INTRODUCTION

The leaves of *Blumea balsamifera* (L) DC commonly called "sambong" have been used for arthritis, chest pain, cough and tympanism (Philippine National Formulary, 1980). The many uses of "sambong" are very well documented. It is therefore important to find out if "sambong" tablets prepared from "sambong" leaves are genotoxic.

MATERIALS AND METHODS

Rec assay (Kada *et al,* 1980) was used for determining direct DNA damaging potential. Mutagenicity before metabolic activation was studied using the method of Moriya (1980) and clastogenicity potential was studied using the micronucleus test (Schmid, 1976). Antimutagenic effects were investigated against dimethylnitrosamine, tetracycline and mitomycin C (Schmid, 1976).

RESULTS AND DISCUSSION

The results of the Rec assay are shown in Table 1. No zones of inhibition due to "sambong" were observed in both Rec^+ and Rec^- organisms. This suggests that "sambong" tablets do not possess direct DNA damaging potential.

The results in Tables 2 and 3 show that "sambong" tablets are not genotoxic before metabolic activation. They do not contain base-pair and frameshift mutagens. Revertants per plate are in the same range as the negative control and placebo tablets.

Table 4 shows the results of the host-mediated assay. The mutation frequency due to "sambong" tablets falls within the range of the negative control indicating that "sambong" tablets are not mutagenic after metabolic activation.

The results for clastogenicity potential are given in Table 5. The data show that "sambong" tablets do not fragment the chromatin material of the bone marrow cells and they do not possess chromosome-breaking effects.

Table 6 shows that "sambong" tablets reduce the mutagenicity potential of dimethylnitrosamine which is not only a mutagen but also a carcinogen (Fishbein, 1978). The number of micronucleated polychromatic erythrocytes was greatly reduced by these tablets indicating the antimutagenic activity of these tablets against dimethylnitrosamine. Dimethylnitrosamine is metabolized to an alkylating agent of DNA (Fishbein, 1978). The "sambong" tablets, therefore, reduce the tendency of dimethylnitrosamine to alkylate DNA.

Table 7 shows the reduction of the genotoxicity of tetracycline by "sambong" tablets. Tetracycline is not only a mutagen but also a teratogen (Sylianco and Blanco, 1984). It is genotoxic not only to somatic cells but also to germ cells (Sylianco and Blanco, 1984). Its genotoxicity is a consequence of intercalation and hyrogen bonding interactions with base-pairs of DNA. "Sambong" tablets, by reducing its genotoxicity must have reduced the intercalating and hydrogen bonding capacity of tetracycline with DNA.

Table 8 shows that these tablets reduce the mutagenicity of mitomycin C. The number of micronucleated polychromatic erythrocytes induced by mitomycin C was significantly reduced indicating the antimutagenic activity of these tablets against mitomycin C.

Mitomycin C is metabolized into alkylating intermediates. It was shown to cause a high incidence of chromatic breaks in spermatogonia (Adler, 1974). Mitomycin C also induces dominant lethality (Ehling, 1971). The reduction of micronucleated polychromatic erythrocytes induced by mitomycin C by "sambong" tablets suggests that these tablets reduce the tendency of mitomycin C intermediates to alkylate DNA.

SUMMARY AND CONCLUSIONS

1. "Sambong" tablets from NSTA pilot plant do not possess direct DNA damaging capacity.

2. They are not mutagenic before and after metabolic activation and they do not possess chromosone-breaking effects.

3. However, "sambong" tablets were shown to possess antimutagenic activity against dimethylnitrosamine, tetracycline and mitomycin C by reducing the formation of micronucleated polychromatic erythrocytes induced by these three mutagens.

ACKNOWLEDGEMENT

Financial support from Philippine Council of Health Research and Development is gratefully acknowledged.

REFERENCES

1. Adler, I.D. 1974. Comparative cytogenetic study after treatment of spermatogonia with mitomycin C. *Mutat. Res.* **23**: 369-379.
2. Ames, B.N. 1971. The detection of chemical mutagens by enteric bacteria. *Chemical Mutagens* **1**: 267-282.
3. Ehling, U.H. 1971. Comparison of radiation and chemically induced dominant lethal mutations in male mice. *Mutat. Res.* **11**: 35-44.
4. Fishbein, L. 1978. Naturally Occurring mutagens. *Mutagenesis* 1975-231, Hemisphere Publishing Company, London.
5. Kada, T., K. Hirano and Y. Shirazu. 1980. Detection of environmental mutagens by the Rec assay. *Chemical Mutagens* **6**: 149-173.
6. Moriya, M. 1980. Workshop Manual. Southeast Asian Conference on Environmental Mutagens, Carcinogens and Teratogens, 14-17.
7. Philippine National Formulary. 1980. NSTA, Bicutan p. 82.
8. Schmid, W. 1975. The micronucleus test. *Mutat. Res.* **31**: 9-15.
9. Sylianco, C.Y.L., and F.R.B. Blanco. 1984. Effects of vitamin C and E on the teratogenic potential of tetracycline. *Nat. and App. Sci. Bull.* **36**: 1-6.

Table 1. DNA-damaging potential of "sambong" tablets.

	Zone of inhibition (mm)* Rec$^+$	Rec$^-$
Positive control (AF-2)	11.92 ± 2.97	31.92 ± 6.63
Negative control (H_2O)	0	0
Placebo tablet **	0	0
"Sambong", 7.5 mg/kg	0	0
15 mg/kg	0	0
30 mg/kg	0	0

* Zone of inhibition values based on 15 plates
** Placebo tablet contains starch and magnesium stearate.

Table 2. Mutagenicity potential before metabolic activation of "sambong" tablets using *S. typhimurium* T.A. 1537

	Revertants per plate
Positive control (9-aminoacridine)	TNTC**
Distilled water	8,12 ± 0.98
Placebo tablet	6.88 ± 1.40
"Sambong", 7.5 mg/kg	6.24 ± 1.20
" 15 mg/kg	6.80 ± 0.98
30 mg/kg	7.80 ± 2.34

**Too numerous to count

Table 3. Mutagenicity potential before metabolic activation of "sambong" tablets using *S. typhimurium* TA 1537.

	Revertants per plate*
2 aminoflourene	TNTC**
Distilled water	16.00 ± 2.20
Placebo tablet	15.32 ± 3.24
"Sambong", 7.5 mg/kg	12.40 ± 3.70
15 mg/kg	10.88 ± 3.60
30 mg/kg	12.16 ± 4.22

*average of 15 plates
**Too numerous to count

Table 4. Mutagenicity potential after metabolic activation of "sambong" tablets using the host-mediated assay.

	Mutation Frequency *
Dimethylnitrosamine, 15 mg/kg	7.26 ± 1.14
Distilled water	0.91 ± 0.14
Placebo tablet	0.83 ± 0.38
"Sambong", 7.5 mg/kg	0.76 ± 0.59
15 mg/kg	1.48 ± 0.51
30 mg/kg	1.45 ± 0.31

*Mutation frequency of *S. typhimurium* G 46 based on 15 plates

Table 5. Clastogenicity potential of "sambong" tablets using the micronucleus test.

	No. of micronucleated polychromatic erythrocytes per thousand*
Dimethylnitrosamine, 15 mg/kg	12.45 ± 1.23
Distilled water	2.07 ± 0.43
Placebo tablet	0.80 ± 0.65
"Sambong", 7.5 mg/kg	0.87 ± 0.32
15 mg/kg	1.00 ± 0.23
30 mg/kg	1.13 ± 0.38

*average value of 15 slides

Table 6. Antimutagenic effects of "sambong" tablets against dimethylnitrosamine (DMN).

	No. of micronucleated polychromatic erythrocytes per thousand
Dimethylnitrosamine, 15 mg/kg	12.45 ± 1.23
DMN plus "sambong", 7.5 mg/kg	3.07 ± 0.67
15 mg/kg	2.13 ± 0.76
30 mg/kg	0.87 ± 0.12
Control	1.55 ± 0.46

Table 7. **Antimutagenic effects of "sambong" tablets against tetracycline (Tet).**

	No. of micronucleated polychromatic erythrocytes per thousand
Tetracycline, 55 mg/kg	13.50 ± 0.63
Tet plus "sambong", 7.5 mg/kg	2.87 ± 0.77
15 mg/kg	1.27 ± 0.34
30 mg/kg	1.27 ± 0.49
Control	1.45 ± 0.32

Table 8. **Antimutagenic effects of "sambong" tablets against mitomycin C (MMC).**

	No. of micronucleated polychromatic erythrocytes per thousand
Mitomycin C, 3 mg/kg	9.41 ± 1.90
MMC plus "sambong", 7.5 mg/kg	2.27 ± 0.43
15 mg/kg	2.08 ± 0.63
30 mg/kg	0.93 ± 0.06
Control	1.34 ± 0.09

THE WILD VERTEBRATE FAUNA OF MOUNT MAKILING, LAGUNA

CORAZON CATIBOG-SINHA
Department of Biology
De La Salle University
Taft Avenue, Manila

ABSTRACT

A total of 296 specimens represented by 5, 10, 28, and 11 species of amphibians, reptiles, aves and mammals, respectively, were collected from the northeast section of Mt. Makiling, Laguna, during the period January-December 1985. Only those species with positive identification are included in this paper. Data on body weight and measurements of birds and mammals, grouped by sex and age classes, are also incorporated in this report.

INTRODUCTION

Mt. Makiling, known to be a dormant volcano, was established as a National Forest Reserve in 1910. It is about 200 km^2 in area and straddles between the provinces of Batangas and Laguna. It lies within 121°08′ and 121°18′ east longitude and 145°05′ and 145°15′ north latitude. It is bounded in the north by the campus of the University of the Philippines at Los Baños. Its altitude ranges from 100 m to about 1000 m above sea level. Several streams and creeks traverse this rugged mountains, notably the Calo, Molawin, Pili, and Dampalit Creeks.

Despite the popularity of Mt. Makiling as a protected forest reserve, very little information about its vertebrate fauna has been documented except for some collection records deposited with the UPCA and UPCF museums and/or laboratories. This study therefore aims to document the species of amphibians, reptiles, aves and mammals which had been collected from the northeast section of Mt. Makiling in 1986. The list also provides quantitative data on the animal's morphological features, such as body weight and external measurements to serve as identification guide for future taxonomic work.

Pancho (1983) gives a comprehensive review of Mt. Makiling and a description of its physical and biotic environment. Alviola (1977) was able to record 120 avian species in Mt. Makiling as well as in the UPLB lowland campus. Of the total species of birds recorded, Alveola reported 30 new records. Mendoza *et al,* (in press) who studied the horizontal distribution of birds from Mayondon, Laguna to the UPCF campus observed 31 species under 23 families of which 4 new species were recorded. Mendoza (1986) proposed some guidelines in the census of forest birds using Mt. Makiling as the model site. Taylor (1922, 1934) did some inventory of the amphibians, reptiles and mammals in Mt. Makiling. The big mammals previously identified in the area are the wild pig *(Sus celebensis),* deer *(Cervus philippinus),* monkey *(Macaca philippinensis),* palm civet *(Paradoxurus philippinensis)* and Malay civet *(Viverra tangalunga).*

However, none of the above-mentioned studies reported on the morphometric aspect of the wild vertebrate fauna of Mt. Makiling.

METHODS

The collection site was established in the northern section of Mt. Makiling, specifically on or adjacent to the UPCF campus and experimental forest, located at 100-350 m above sea level (Fig. 1). Collection started in January and ended in December 1985. Less intensive collection effort was exerted during the last quarter of the year when the species diversity of the wild fauna became more or less constant. Mist nets were used in the collection of birds and bats while 150 x 250 cm wire traps were used in the collection of rodents; amphibian and reptilian specimens were hand-grabbed.

Body weights of birds and mammals were determined in the field using a 25-g portable spring balance. Standard body measurements of mammals were also measured using an ordinary ruler. Sex and age of all collected specimens were determined based on morphological features,, i.e., type and size of gonads, plumage/fur/skin coloration. All collected birds and mammals were stuffed using the standard method of preparing museum specimens while the amphibians and reptiles were preserved in 10% formalin.

A tabulation of the above data for each species grouped by family and arranged according to Dupont's (1971) sequence of taxonomic listing was prepared. The mean, range and standard deviations of the body weights and measurements of birds, bats, and rats were also computed and presented in the tables by age and sex per species.

RESULTS AND DISCUSSION

A total of 121 adult and 5 immature birds under 28 species and 18 families were collected as museum specimens (Table 1). The Philippine "bulbul" *(Hypsipetes philippinus)* was the most frequently observed and caught forest bird (capture frequency = 27.78%) in the study area, followed by the yellow-bellied whistler *(Pachycephala p. philippinensis)* with a capture frequency of 9.52%. *H. philippinus* is very common throughout the Philippines (Rabor, 1977) and was also the most captured species in Twin Lakes, Balinsasayao, Negros Oriental (Erickson and Heideman, 1983).

The species not included in Alviola's (1977) avifaunal list and may be considered as new records in Mt. Makiling are the following: (1) black-chinned fruit dove *(Ptilinopus lechlancheri)* (2) black-crowned tree babbler *(Stachyris capitalis affinis)* (3) Dubois' leaf warbler *(Phylloscophus cebuensis)*, (4) plain-throated sunbird *(Anthreptes malacensis birgitae)* (5) white-bellied flowerpecker *(Dicaeum hypoleucum obscurum)*, and (6) yellow-white eye *(Zosterops nigrorum)*.

In general male birds, regardless of species, were more frequently caught than females. Likewise, adult males tend to be heavier than adult female birds.

Table 2 shows a listing of bats collected from the study area. This collection includes 8 species, 4 families and 130 specimens of bats. Family Pteropidae was the most represented family in terms of number of species (5) and composition (113 specimens).

The 3 most frequently captured bat species in the area were the Geoffroy's rousette bat *(Rousette amplexicaudatus)* and the shortnose fruit bats *(Ptenochirus jagori* and *Cynopterus brachyotis*), with capture frequencies of 35.38%, 23.08%, and 19.23%, respectively. All these are widespread in the Philippines (Rabor, 1977). *C. brachyotis* is known to be widely distributed in Southeast Asia and found to be most abundant in all habitat types in Camiguin Island (Heaney, 1984) and Lake Balinsasayao Region (Heaney *et al,* 1981).

The 5 other bat species were captured 1-14 times only during the entire collection period. The extreme variations in capture frequencies and species abundance in tropical bats were also noticed by Heaney *et al* (1981) and Fleming (1975).

Unlike bats which represented the bulk of mammalian collection, forest rats consisted only of 8 specimens belonging to 3 species under Family Muridae (Table 3). *Rattus r. mindanensis* (Philippine field rat) was the most commonly occurring non-volant land mammal, and usually captured in dense vegetation.

Table 4 is a listing of collected amphibians (5 species, 3 families, 19 specimens) and reptiles (10 species, 7 families, 13 specimens) from the study area. No attempt was made to classify these specimens by sex or age.

ACKNOWLEDGEMENT

The author would like to acknowledge the Science Foundation of the De La Salle University, Taft Ave., Manila, for providing the research funding; Mr. Esmeraldo Macatiguib and Ms. Lucita M. Exconde for their assistance in the implementation of the study, Ms. Marites M. Humarang for typing the manuscript, and Ms. Cecille Taguiam for helping in preparing the tables.

REFERENCES

1. Alcasid, G.L. 1970. Checklist of Philippine mammals. National Museum, Department of Education (Manila). Museum Pub. No. **5**:1-51.
2. Alviola, P.L. III. 1977. Avifauna of Mt. Makiling. MS Thesis. University of the Philippines at Los Baños, 156 pp.
3. Catibog-Sinha, C. 1982. The birds and mammals of MUFRC experimental forest. *Sylvatrop Philipp. For. J.* 7(1):35-42.
4. Dupont, E. 1971. Philippine birds. Monograph Series No. 2. Delaware Museum of National History, Delaware 479 pp.
5. Dupont, J.E., and D.S. Rabor. 1973. Birds of Dinagat and Siargo, Philippines, an expedition report. *Nemouria* (Occasional papers of the Delaware Museum of Natural History) **10**:1-11.

6. Erickson, K.R., and P.D. Heideman. 1983. Notes on the avifauna of the Balinsasayao rainforest region, Negros Oriental, Philippines. *Siliman J.* **30** (1-2): 63-72.
7. Fleming, T.H. 1975. The role of small mammals in tropical ecosystems. pp. 269-298. *In* F.B. Golley, K. Petrusawics, and L. Ryskowski (Eds.), Small mammals, their productivity and population dynamics. Cambridge University Press.
8. Heaney, L. R. 1984. Mammals from Camiguin Island, Philippines. *Proc. Biol. Soc. Wash.* **97**(1): 119-125.
9. Heaney, L.R., P.D. Heideman and K.M. Mudar. 1981. Ecological notes on mammals in the Lake Balinsasayao Region, Negros Oriental, Philippines. *Siliman J.* **28** (3-4): 122-131.
10. Heaney, L.R., and D.S. Rabor. 1982. Mammals of Dinagat and Siargao Islands, Philippines. Occasional Papers of the Museum of Zoology, U. Michigan, No. **699**:1-30.
11. Mendoza, M.M. 1986. Optimal time and duration of avian census in a forested area. M.S. Thesis. University of the Philippines at Los Baños.
12. Mendoza, M., V. Phan Quang and C. Custodio. 1986. Avifaunal variation from the lakeshore to the forest edge of Mt. Makiling in Los Baños, Laguna. *Sylvatrop Philipp. For. Res. J.* In press.
13. Pancho, J.V. 1983. Vascular flora of Mt. Makiling and vicinity (Luzon: Philippines), Part I. *Kalikasan, The Philipp. J. Biol.* Supplement No. 1. New Mercury Printing Press, Q.C. 476 pp.
14. Rabor, D.S. 1977. Philippine Birds and Mammals. Univ. Philippines Press, Q.C. 284 pp.
15. Sanborn, C.C. 1952. Philippine zoological expedition 1946-47: *Mammals, Fieldiana: Zoology.* **33**(2): 89-158.
16. Taylor, E.H. 1922. Herpetological fauna of Mt. Makiling. *Philipp. Agric.* **11** (15): 125-140.
17. Taylor, E.H. 1934. Philippine Land Mammals Bureau of Printing, Manila. Monograph No. 30:548.

Table 1. Body weights of birds collected from Mt. Makiling, January-December 1985.[a]

Family/Species	N	Sex	Body wt. (g) ± S.D. (range)
COLUMBIDAE			
Phapitreron l. leucotis (White-Eared Brown Fruit Dove)	1	M	120
Ptilinopus l. leclancheri (Black-Chinned Fruit Dove)	2	F	147.5 + 3.53 (145-150)
STRIGIDAE			
Ninox p. philippensis (Philippine Boobook Owl)	1	F	115
ALCEDINIDAE			
Halcyon l. lindsayi (Spotted Wood Kingfisher)	1	M	80
DICRURIDAE			
Dicrurus b. balicassius (Balicassiao)	4	M	68.87 ± 2.59 (65-70.5)
	1	F	70
CAPITONIDAE			
Megalaima h. haemacephala (Crimson-Breasted Barbet)	1	M*	35
PARIDAE			
Parus e. elegans (Elegant titmouse)	6	M	16.75 ± 4.17 (10-20.5)
RHABDORNITHIDAE			
Rhabdornis m. mysticalis (Striped-Headed Creeper)	2	M	22 ± 1.41 (21-23)
	2	F	20 ± 0
TIMALIIDAE			
Stachyris capitalis affinis (Black-Crowned Tree Babbler)	1	F	20
PYCNONOTIDAE			
Hypsipetes p. philippinus (Philippine Bulbul)	20	M	36.25 ± 4.83 (30-50)
	15	F	36.27 ± 4.46 (30-42)
Pycnonotus g. goiavier (Yellow-Vented Bulbul)	2	M	35.0 ± 7.07(30-40)
	2	F	35.5 ± 7.78 (30-41)
P. u. urostictus (Wattled Bulbul)	4	M	27.5 ± 5.0 (20-30)
	2	F	25.0 ± 7.07 (20-30)
TURDIDAE			
Copsychus l. luzoniensis (White-Eyebrowed Shama)	4	M	28.75 ± 2.5 (20-30)
	3	F	22.33 ± 4.04 (20-27)

Table 1 (Continued)

Family/Species	N	Sex	Body wt. (g) ± S.D. (range)
SYLVIIDAE			
Phylloscophus cebuensis (Dubois' Leaf Warbler)	1	M	10
MUSCICAPIDAE			
Hypothymis a. azurea (Black-Naped Blue Monarch)	1	M	10
	1	F	15
Pachycephala p. philippinensis (Yellow-Bellied Whistler)	8	M	25.94 ± 5.44 (20-35)
	4	F	22.50 ± 5.00 (20-30)
Rhipidura c. cyaniceps (Blue-Headed Fantail)	4	M	16.25 ± 4.79 (10-20)
	5	F	13 ± 4.47 (10-20)
LANIIDAE			
Lanius cristatus lucionensis (Brown Shrike)	3	F	26.33 ± 5.51
NECTARINIIDAE			
Anthreptes malacensis birgitae (Plain-Throated Sunbird)	1	F	15
Nectarinia j. jugularis (Olive-Backed Sunbird)	1	M	10
DICAEIDAE			
Dicaeum a. australe (Philippine Flowerpecker)	2	M	9.5 ± 0.71 (9-10)
	2	M*	6.5 ± 0.71 (6-7)
	2	F*	9.0 ± 1.41 (8-10)
D. hypoleucum obscurum (White-Bellied Flowerpecker)	1	M	10.0
D. p. pygmaeum (Pygmy Flowerpecker)	1	M	7.0
D. trigonostigma (Orange-Breasted Flowerpecker)	2	M	6.5 ± 0.71 (6-7)
ZOSTEROPIDAE			
Zosterops m. meyeni (Philippine White-eye)	1	F	10
Zosterops nigrorum luzonica (Yellow White-eye)	3	M	7.33 ± 1.53 (6-9)
	3	F	8.33 ± 0.58 (8-9)
PLOCEIDAE			
Passer montanus saturatus (Tree Sparrow)	2	M	14.0 ± 2.83 (12-16)
ESTRILDIDAE			
Lonchura leucogastra manueli (White-Breasted Mannikin)	2	M	15.0 ± 7.07 (10-20)
	2	F	15 ± 7.07 (10-20)

[a] All are adult birds except those marked with asterisk (*)
N = sample size, F = female, M = male

Table 2. Weight and body measurements of bats collected from Mt. Makiling, January-December 1985.[a]

Family/Species	N	Body Weight (g)	Body Measurements (mm) Total	Tail	Ear	Hindfoot
PTEROPIDAE						
Cynopterus brachyotis (Short-Nosed Fruit Bat)						
Adult Male	17	35.29 ± 3.74 (30-40)	102.12 ± 2.12 (95-105)	8.94 ±1.52 (6-11)	12.59 ± 3.59 (13-18)	16.12 ± 1.22 (14-17)
Immature Male	1	30	95	9	16	15
Adult Female	7	37.2 ± 11.01 (25-50)	102.71 ± 3.35 (100-109)	8.71 ± 0.95 (7-10)	15.71 ± 1.38 (13-17)	15.57 ± 1.90 (12-17)
Ptenochirus jagori (Short-Nosed Fruit Bat)						
Adult Male	17	62.94 ± 5.88 (50-70)	122 ± 5.34 (106-129)	10.82 ± 1.42 (9-14)	18.65 ± 1.06 (16-20)	19.53 ± 0.94 (18-21)
Immature Male	2	42.5 ± 10.61 (35-50)	111.5 ± 9.19 (105-118)	8.5 ± 0.71 (8-9)	17.0 ± 1.41 (16-18)	18.0 ± 0 (18-18)
Adult Female	10	73 ± 12.52 (50-90)	127.8 ± 4.52 (121-134)	10.9 ± 1.45 (9-14)	18.8 ± 1.40 (16-21)	19.2 ± 0.92 (18-21)
Immature Female	1	50	119	11	19	20
Rousettus amplexicaudatus (Geoffroy's Rousette Bat)						
Adult Male	16	56.81 ± 11.31 (40-80)	133.37 ± 8.86 (115-149)	17.37 ± 2.80 (13-20)	18.44 ± 0.81 (16-19)	20.06 ± 0.57 (19-21)
Immature Male	1	30	111	11	17	18
Adult Female	26	69.50 ± 11.97 (40-95)	137.61 ± 6.05 (124-147)	16.15 ± 1.84 (11-20)	18.35 ± 1.67 (11-21)	19.77 ± 1.07 (18-21)
Immature Female	3	50.33 ± 4.51 (46-55)	126.67 ± 6.11 (120-132)	15.33 ± 0.58 (15-16)	18.33 ± 0.58 (18-19)	19.67 ± 0.58 (19-20)

Eonycteris spelaea (Dawn Bat)						
Adult Male	3	65 ± 13.23 (50-75)	126.67 ± 2.08 (125-129)	14.33 ± 3.21 (12-18)	19.67 ± 1.53 (18-21)	19.33 ± 2.52 (17-22)
Adult Female	1	125	15	18	20	70
Immature Female	2	42.5 ± 3.53 (40-45)	110.5 ± 6.36 (106-115)	12.5 ± 2.12 (11-14)	18 ± 1.41 (17-19)	18.5 ± 0.71 (18-19)
Macroglossus lagochilus (Long-Tongued Fruit Bat)						
Adult Male	1	20	68	0	12	12
Adult Female	5	15.6 ± 5.13 (10-20)	72.40 ± 2.19 (69-75)	0	13 ± 1.22 (12-15)	11.4 ± 0.89 (11-13)
MEGADERMATIDAE						
Megaderma sp. (False vampire)						
Adult Female	1	15	96	0	40	19
HIPPOSIDERIDAE						
Hipposideros diadema (Leaf-Nosed Bat)						
Adult Female	2	4.25 ± 3.54 (40-45)	138.5 ± 3.53 (136-141)	39.5 ± 6.36 (35-44)	25 ± 0 (25 ± 25)	17.5 ± 2.12 (16-19)
VESPERTILIONIDAE						
Scotophilus timmincki (Yellow-Brown Bat)						
Adult Male	4	17 ± 2.83 (15-21)	121 ± 3.56 (118-126)	46.25 ± 0.5 (46-47)	13.25 ± 2.06 (11-16)	10.5 ± 0.58 (10-11)
Immature Male	1	10	105	40	12	11
Adult Female	8	17 ± 3.78 (15-21)	119.75 ± 6.30 (114-129)	45.25 ± 2.12 (42-48)	13.75 ± 1.03 (13-15)	10.62 ± 0.52 (10-11)
Immature Female	1	10	115	45	13	11

[a] Values presented are means ± standard deviation (range)

Table 3. Weight and body measurements of forest rats (Family Muridae, Order Rodentia) collected from Mt. Makiling, January-December, 1985.*

Species	N	Body Weight (g)	Body Measurements (mm)			
			Total	Tail	Ear	Hindfoot
Rattus norvegicus						
(Norway Rat)						
Adult male	1	230	381	175	20	42
Rattus everetti						
(Everett's Rat)						
Immature male	1	150	336	198	21	40
Adult Female	2	245 ± 35.35 (220-270)	414.5 ± 57.28 (374-455)	196 ± 70.71 (146-246)	24 ± 0 (24-24)	45 ± 1.41 (44-46)
Rattus r. mindanensis						
(Philippine Field Rat)						
Adult male	2	100 ± 28.28 (80-120)	379.5 ± 57.28 (339-420)	175 ± 7.07 (170-180)	19 ± 0 (19-19)	36.5 ± 2.12 (35-38)
Immature male	1	90	306	158	18	35
Adult Female	1	120	316	166	18	38

*Values presented are means ± standard deviation (range).

Table 4. Amphibians and reptiles collected from Mt. Makiling, January-December 1985.

	No. of Specimens
I. Amphibians	
Order Anura	
1. Family Ranidae	
Ooeidozyga laevis laevis	12
Platymantis (Cornufer) meyeri	2
Rana sp.	2
2. Family Rhacophonidae	
Rhacophorus leucomystax	1
3. Family Microphylidae	
Kaloula picta	2
Total	19
II. Reptiles	
Order Squamata	
SUBORDER SAURIA – LIZARDS	
1. Family Gekkonidae – Geckos	
Gecko gecko gecko	2
2. Family Agamidae – Flying lizards	
Draco volans	1
3. Family Scincidae – Skinks	
Mabuya multifasiata	1
Sphenomorphus sp.	1
SUBORDER OPHIDIA – SNAKES	
1. Family Natricidae	
Cyclocorus lineatus	1
Lycodon aulicus	1
Dendrophis pictus	2
2. Family Boigidae	
Chrysopelea paradisii	2
3. Family Elapidae – cobras koraits	
Calliophis calligaster	1
4. Family Crotalidae – Pet Vipers	
Trimeresurus flavomaculatus	1
Total:	13

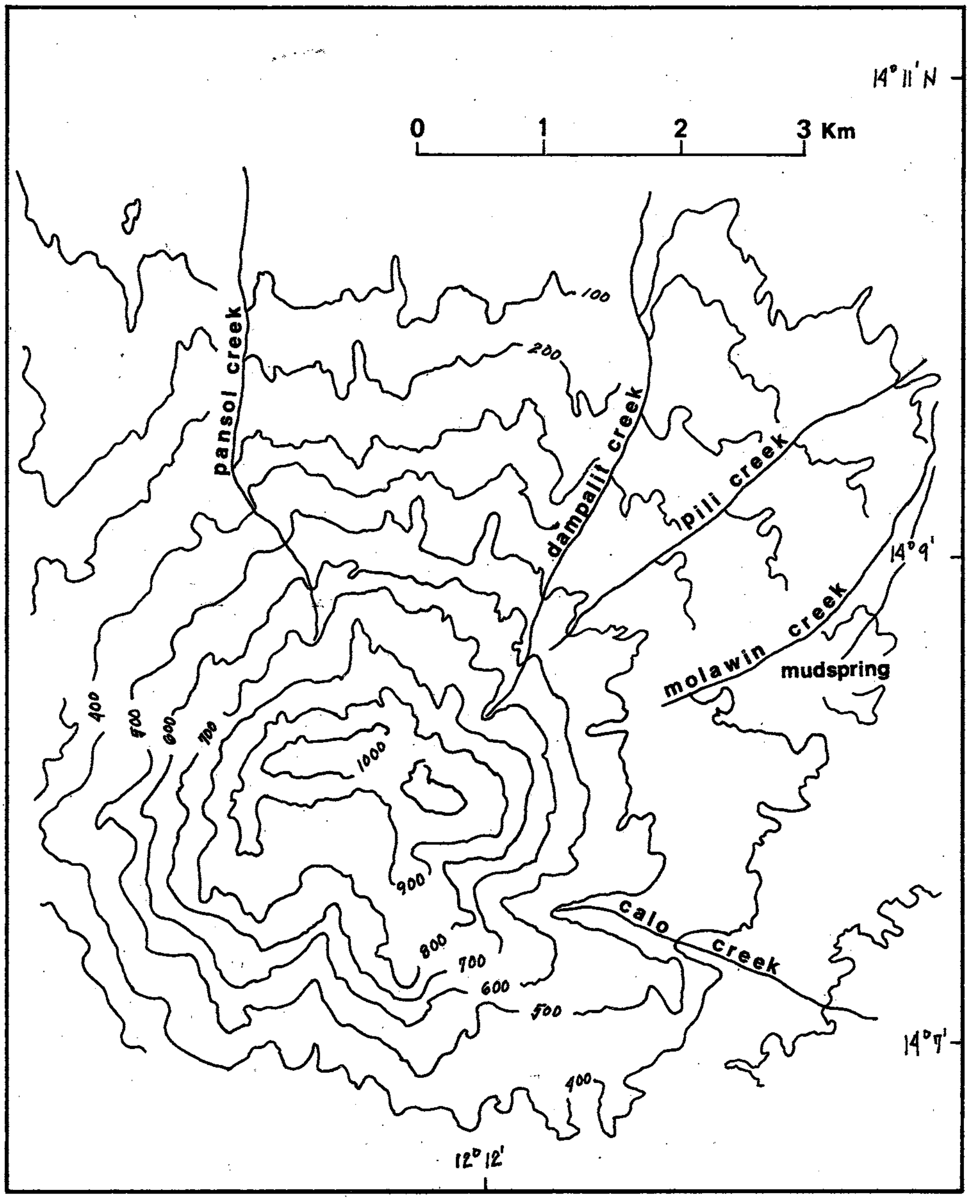

Figure 1. A portion of Mt. Makiling

UPDATED LIST OF MT. MAKILING AVIFAUNA

MARLYNN M. MENDOZA
Forest Research Institute
College, Laguna

ABSTRACT

One hundred and thirty-two species of birds belonging to 15 orders, 49 families and 101 genera have so far been identified in Mt. Makiling and vicinities. Twelve are new records in the area.

INTRODUCTION

The most recent checklist of birds found in Mt. Makiling in Laguna is that of Alviola (1977). He compiled his list from four sources, namely: (a) original records from literature; (b) 1964-1966 bird banding records of the Migratory Animals Pathological Survey (MAPS) in Mt. Makiling by the personnel of the Philippine National Museum; (c) Department of Zoology, College of Arts and Sciences, University of the Philippines at Los Baños Museum record and; (d) collection and visual observation records of birds from his survey (November 1971 to October 1972). Alviola (1977) listed 120 species of birds.

In 1986, two studies on birds were conducted in Mt. Makiling. Mendoza, Custodio and Phan (1986) investigated the avifaunal variation from the lakeshore of Laguna de Bay to the forest edge of Mt. Makiling and Mendoza (1986) conducted a study on the optimum time and duration of avian census in a forested area. Twelve new species were recorded in addition to Alviola's checklist as a result of the two studies made.

The twelve new bird records are listed below with their description, measurements and some notes. The descriptions and measurements (in millimeters) of the birds are based on duPont (1971) and Delacour and Mayr (1946).

Wandering Whistling Duck (Plate 1)

Dendrocygna arcuata arcuata (Horsfield, 1824)

Order: Anseriformes

Family: Anatidae

Description: Top of head dark brown; back, wings and tail black; wing-coverts rufous; feathers of mantle edged dark fulvous; chin and throat dirty white; breast fulvous with black spots, belly pale cinnamon; under tail-coverts dirty white; beak black; iris brown; feet gray-black.

Measurements: Wing 203; tail 68; beak 46; tarsus 48.

Notes: They were once seen in the morning of September 1981 flying over the Laguna de Bay in Mayondon, Los Baños (Mendoza *et al,* 1986).

The food of these birds includes plants, mostly aquatic, mollusks, worms, frogs, fishes, reptiles and to a certain extent insects and larvae (Rabor, 1977).

It is found throughout the Philippines.

Black-chinned Fruit Dove (Plate 2)

Ptilinopus leclancheri leclancheri (Bonaparte, 1855)

Order: Columbiformes

Family: Columbidae

Description: Head, neck, throat and breast light gray; nape somewhat greenish; upper parts green; chin black; wide brownish maroon band separates the breast from all green belly; under tail-coverts cinnamon. Generally, beak yellow; iris red; feet dark red.

Measurements: Wing 144; tail 110; beak 15; tarsus 21.

Notes: A forest species observed once at the forest edge of Mt. Makiling, 220 m above sea level. It was seen in the morning of November 1984 (Mendoza, 1986).

A bird was collected by Macatiguib (pers. comm.) and the specimen is in the possession of Dr. C. Sinha of the Outdoor Recreation and Wildlife Research Division of the Forest Research Institute.

Crimson-backed Woodpecker (Plate 3)

Chrysocolaptes lucidus haematribon (Wagler, 1827)

Order: Piciformes

Family: Picidae

Description: Male-head, back and wings dark red; tail brown; throat and breast black with white spots; under-parts pale buff with brown bars. Female-similar to the male but with black head spotted with white. Generally, beak black; iris red; feet dark green.

Measurements: Wing 141; tail 90; beak 40; tarsus 27.

Notes: An endemic bird that flies from one tree to another in search for food. It can be found from 200 to 2000 m elevation (Rabor, 1968).

In Mt. Makiling, it was observed several times near Flatrocks in June 1983 and November 1984 (Mendoza 1986).

Black-crowned Tree Babbler (Plate 4)

Stachyris capitalis affinis (McGregor, 1907)

Order: Passeriformes

Family: Timaliidae

Description: Forehead and crown black; nape grayish chestnust; generally upperparts more olive than *S. c. nigrocapita;* back grayish with light shaft streaks; wings and tail dark olive-brown; outer rectrices tipped with white; cheeks grayish; throat yellow with rufous; underparts heavily washed with olive-yellow; beak black; iris red-brown; feet olive-brown.

Measurements: Wing 68; tail 60; beak 16; tarsus 20.

Notes: It is usually found in molave and dipterocarp forests from 200 to 800 m (Rabor, 1968).

The bird was observed in Mt. Makiling at 250 m above sea level in November 1984 (Mendoza, 1986).

Yellow-breasted Wren-warbler (Plate 5)

Gerygone sulphurea simplex Cabanis, 1872

Order: Passeriformes

Family: Sylviidae

Description: Upper parts dark olive-brown; lores white; underparts yellowish; beak and feet black; iris dark brown.

Measurements: Wing 52; tail 35; beak 12; tarsus 16.

Notes: A bird of the open country, in tree tops, mangroves, bamboo clumps and native gardens (Delacour and Mayr, 1946; Rabor, 1968).

It was observed in bamboo thickets in Mayondon, Los Baños, Laguna. This endemic species produces a pleasing call (Mendoza *et al* 1986).

Philippine Leaf Warbler (Plate 6)

Phylloscopus olivaceus (Moseley, 1891)

Order: Passeriformes

Family: Sylviidae

Description: Upperparts olive-green; rump lighter; superciliary yellow; chin and throat whitish; breast and belly gray-white washed with yellow; under tail-coverts yellow; beak, upper mandible black, lower mandible yellowish; iris brown; feet blackish.

Measurements: Wing 60; tail 50; bill 14; tarsus 18.

Notes: A bird that inhabits the lowland forest (Delacour and Mayr, 1946). It was observed several times in June 1983 and November 1984 near Flatrocks in Mt. Makiling at 220 m above sea level (Mendoza, 1985).

Little Pied Flycatcher (Plate 7)

Ficedula westermanni westermanni (Sharpe, 1888)

Order: Passeriformes

Family: Muscicapidae

Description: Male – crown, sides of head and back black; wings black with a white stripe; tail central rectrices black, remainder basal half white, terminal half black; superciliary stripe and underparts white. Female – upperparts gray; tail brownish; under parts white washed with gray. Generally, beak and feet black; iris dark brown.

Measurements: Wing 58; tail 40; beak 11; tarsus 14.

Notes: It was observed once in November 1984 near Flatrocks (Mendoza, 1986). This bird is an endemic species.

Verditer Flycatcher (Plate 8)

Muscicapa panayensis nigrimentalis (Ogilvie-Grant, 1894)

Order: Passeriformes

Family: Muscicapidae

Description: Upperparts verditer-blue; lores black; forehead and chin black, edges of primaries and under parts more silvery verditer-blue; lower belly and under tail coverts white; beak black; iris and feet dark brown.

Measurements: Wing 79; tail 68; beak 13, tarsus 16.

Notes: It is an endemic species and usually found on tree tops (Delacour and Mayr, 1946). According to Rabor (1968), the bird can be found at zone 3 (800 to 1800 m) but may extend the vertical distribution into the upper limits of zone 2 (200 to 800 m) and into the lower limits of zone 4 (1800 to 2000 m).

The species was observed once in November 1984 near Flatrocks at an elevation of 210 m above sea level (Mendoza, 1986).

Plain-throated Sunbird (Plate 9)

Anthreptes malacensis birgitae (Salomonson, 1953)

Order: Passeriformes

Family: Nectariniidae

Description: Male – top of head and upper back metallic green; sides of face red; chin and throat gray; under parts dark with dull olive green; tail dark brown; wings brown edgings greenish or faintly orange, bend metallic purple and ends of scapulars reddish. Female – upperparts dull olive-gray; underparts dark and dull olive-green, generally, beak black; iris brick-red; feet olive-green.

Measurements: Wing 65; tail 49; beak 18; tarsus 16.

Notes: It frequents lowland coconut plantations, gardens and forest edges up to 1000 m (Delacour and Mayr, 1946). It is fairly common at 0 to 800 m and uncommon at 800 to 1800 m (Rabor, 1968).

The bird was seen several times at the forest edge of Mt. Makiling in June 1983 and November 1984 (Mendoza, 1986). Its call was recorded in August 1985 when Mr. C. Custodio of the Outdoor Recreation and Wildlife Research Division of the Forest Research Institute and the author were recording bird calls in Mt. Makiling.

Lovely Sunbird (Plate 10)

Aethopyga shelleyi flavipectus (Ogilvie-Grant, 1894)

Order: Passeriformes

Family: Nectariniidae

Description: Male – crown metallic purplish green; hind neck and upper back dark red; lower back and rump bright yellow; upper tail coverts and tail metallic blue-green; sides of head dark red; chin and throat yellow with a red margin followed by a peacock blue-green malar stripes; breast yellow with red (non-existent or only slightly traceable); belly pale gray. Female – upper parts olive-green; under parts grayish yellow. Generally, beak and feet black; iris dark brown.

Measurements: Wing 46; tail 45; beak 16; tarsus 14.

Notes: The species lives in molave and dipterocarp forests (Rabor, 1968). It was observed several times in the forest edge of Mt. Makiling in June 1983 and November 1984 (Mendoza, 1986).

The bird is an endemic species.

Flaming Sunbird (Plate 11)

Aethopyga flagrans flagrans (Oustalet, 1876)

Order: Passeriformes

Family: Nectariniidae

Description: Male – forehead and forecrown metallic blue-green; hind crown; back and wing coverts bright yellow-olive washed with orange; upper tail-coverts metallic blue-green; tail black with metallic green edges; chin dark metallic purple; throat and breast dull black with a bright reddish orange strip in the middle extending to the dentral part of belly; rest of belly and flanks olive-yellow; under tail-coverts whitish. Female – upper parts olive-green; under parts grayer with a yellowish patch in the breast. Generally, beak and feet black; iris brown.

Measurements: Wing 49; tail 29; beak 18; tarsus 14.

Notes: It is a lowland bird observed at the UP College of Forestry campus and Flatrocks. The call of this bird was recorded several times in the study headed by Mr. C. Custodio of the Outdoor Recreation and Wildlife Research Division of the Forest Research Institute and assisted by the author.

A specimen collected by Mr. E. Macatiguib is deposited at the UPCF Museum of Natural History (Macatiguib pers. comm.).

White-bellied Flowerpecker (Plate 12)

Dicaeum hypoleucum obscurum (Ogilvie-Grant, 1894)

Order: Passeriformes

Family: Dicaeidae

Description: Upper parts olive-brown; tail and primaries dark brown and underparts grayish olive; beak black; iris brown; feet brownish.

Measurements: Wing 55; tail 27; beak 12; tarsus 14.

Notes: This endemic species is usually found in the forests and open areas from 200-800 m elevation (Rabor, 1968).

It is a common bird at the forest edge of Mt. Makiling. An on-going study on the recording of bird calls in Mt. Makiling was able to record its call.

Macatiguib (pers. comm.) was able to collect the bird and the specimen is with Dr. C. Sinha of the Outdoor Recreation and Wildlife Research Division of the Forest Research Institute.

The addition of 12 species brings the number of bird species found in Mt. Makiling and vicinities to 132 (Table 1). Correspondingly, the additional identified species increased the represented orders in the area from 15 to 16, families represented from 47 to 49 and genera represented from 92 to 101. Of the 12 new species, however, specimens from only three species have been collected, namely, *Ptilinopus l. leclancheri, Aethopyga f. flagrans* and *Dicaeum hypoleucum obscurum.* Specimens of the rest would have to be collected for verification.

ACKNOWLEDGEMENT

The author is grateful to Dr. Phan Quang Vinh, Messrs. Carlo C. Custodio and Esmeraldo Macatiguib for all the help they extended in the preparation of this manuscript and to Mr. Guillermo Villamil for the illustrations.

REFERENCES

1. Alviola III, P.L. 1977. Avifauna of Mt. Makiling. MS Thesis, University of the Philippines, Los Baños, Laguna (Unpublished).
2. Delacour, J., and E. Mayr. 1946. Birds of the Philippines. Macmillan Company. New York.
3. DuPont, J.E. 1971. Philippine Birds. Delaware Museum of Natural History. Delaware.
4. Mendoza, M.M. 1986. Optimal time and duration of avian census in a forested area. MS thesis, University of the Philippines, Los Baños, Laguna (Unpublished).

5. Mendoza, M.M., C.C. Custodio and V.Q. Phan. 1986. Avifaunal variation from the lakeshore to the forest edge of Mt. Makiling, Los Banos, Laguna, *Sylvatrop*. (In Press).
6. Rabor, D.S. 1968. The altitudinal distribution of Philippine birds and mammals. *Proc. Nat'l. Sci. Tech. Week* 13-178.
7. Rabor, D.S. 1977. Philippine Birds and Mammals. University of the Philippine Press. Quezon City.

Table 1. List of Mt. Makiling avifauna

ORDER/ FAMILY/ SCIENTIFIC NAME	Alviola[1] (1977)	Mendoza Custodio and Phan[2] (1986)	Mendoza[3] (1986)
Order Ciconiiformes			
Family Ardeidae			
Ixobrychus s. sinensis (Gmelin, 1789)	d	e	
Ixobrychus cinnamomeus (Gmelin, 1789)	cd		
Order Anseriformes			
Family Anatidae			
Dendrocygna a. arcuata (Horsfield, 1824)		e	
Order Falconiformes			
Family Accipitridae			
Haliastus indus intermedius (Blyth, 1865)	e		e
Haliaeetus leucogaster (Gmelin, 1788)	e		
Spilornis holospilus (Vigors, 1830)	be		e
Accipiter virgatus confusus (Hartert, 1910)	bd	e	
Butastur indicus (Gmelin, 1788)	be		
Family Falconidae			
Microhierax e. erythrogonys (Vigors, 1831)	d		
Order Galliformes			
Family Phasianidae			
Coturnix chinensis lineata (Scopoli, 1786)	bd		
Gallus g. gallus (Linne, 1758)	b	e	e
Family Turnicidae			
Turnix suscitator fasciata (Temminck, 1815)	b		
Order Gruiformes			
Family Rallidae			

Table 1 (Continued)

ORDER/ FAMILY/ SCIENTIFIC NAME	Alviola[1] (1977)	Mendoza Custodio and Phan[2] (1986)	Mendoza[3] (1986)
Rallus s. striatus (Linne, 1766)	bd		
Rallus p. philippensis (Linne, 1766)	bd		
Rallus t. torquatus (Linne, 1766)	bd	e	
Rallina e. eurizonoides (Lafresnaye, 1845)	bd		
Porzana p. pusilla (Pallas, 1776)	bd		
Poliolimnas cinereus ocularis (Sharpe, 1894)	bd	e	
Amaurornis phoenicurus javanicus (Horsfield, 1821)	a		
Gallicrex cinerea (Gmelin, 1789)	bd		
Porphyrio porphyrio pulverulentus (Temminck, 1826)	b		
Gallinula chloropus lozanoi (Lletget, 1918)	bd		
Order Charadriiformes Family Rostratulidae			
Rostratula b. benghalensis (Linne, 1758)	bd		
Family Charadriides			
Pluvialis dominica fulva (Gmelin, 1789)	be		
Charadrius d. dubius (Scopoli, 1786)	e		
Charadrius m. mongolus (Pallas, 1776)	ce		
Family Scolopacidae			
Tringa hypoleucos (Linne, 1758)	b		
Family Laridae			
Chlidonias hybrida javanica (Horsfield, 1821)	d		

Table 1 (Continued)

ORDER/ FAMILY/ SCIENTIFIC NAME	Alviola[1] (1977)	Mendoza Custodio and Phan[2] (1986)	Mendoza[3] (1986)
Order Columbiformes			
Family Columbidae			
Treron pompadora axillaris (Bonaparte, 1855)	e		
Treron v. vernans (Linne, 1771)	b		
Phapitreron l. leucotis (Temminck, 1823)	cd	e	e
Phapitreron a. amethystina (Bonaparte, 1855)	e		
Ptilinopus occipitalis (G.R. Gray, 1844)	e		e
Ducula a. aenea (Linne, 1766)	b		
Ducula p. poliocephala (G.R. Gray, 1844)	b		
Macropygia phasianella tenuirostris (Bonaparte, 1854)	e		e
Streptopelia bitorquata dusumieri (Temminck, 1823)	d		
Streptopelia tranquebarica humilis (Temminck, 1824)	bd		
Geopelia s. striata (Linne, 1766)	bd		
Gallicolumba l. luzonica (Scopoli, 1786)	b		e
Ptilinopus l. leclancheri (Bonaparte, 1855)			e*
Chalcophaps i. indica Linne, 1758)	bcd		e
Order Psittaciformes			
Family Psittacidae			
Bolbopsittacus l. lunulatus (Scopoli, 1786)	bcd		e
Loriculus p. philippensis (P.L.S. Muller, 1776)	bd	e	e
Order Cuculiformes			
Family Cuculidae			

Table 1 (Continued)

ORDER/ FAMILY/ SCIENTIFIC NAME	Alviola[1] (1977)	Mendoza Custodio and Phan[2] (1986)	Mendoza[3] (1986)
Cacomantis m. merulinus (Scopoli, 1786)	e		e
Phoenicophaeus s. superciliosus (Dumont, 1823)	bcd		e
Phoenicophaeus cumingi (Fraser, 1839)	b		e
Eudynamys scolopacea mindanensis (Linne, 1766)	b		
Centropus v. viridis (Scopoli, 1786)	bd		e
Order Strigiformes			
Family Tytonidae			
Tyto capensis amauronota (Cabanis, 1872)	bd		
Family Strigidae			
Otus bakkamoena megalotis (Walden, 1875)	bcd		
Bubo p. philippensis (Kaup, 1851)	bd		e
Ninox p. philippensis (Bonaparte, 1855)	b		e
Order Caprimulgiformes			
Family Caprimulgidae			
Eurostopodus m. macrotis (Vigors, 1831)	b	e	e
Order Apodiformes			
Family Apodidae			
Collocalia esculenta marginata (Salvadori, 1882)	d	e	e
Family Hemiprocnidae			
Hemiprocne comata major (Hartert, 1895)	e		
Family Trogonidae			
Harpactes ardens luzoniensis	e		e
Order Coraciiformes			
Family Alcedinidae			

Table 1 (Continued)

ORDER/ FAMILY/ SCIENTIFIC NAME	Alviola[1] (1977)	Mendoza Custodio and Phan[2] (1986)	Mendoza[3] (1986)
Alcedo atthis bengalensis (Gmelin, 1788)	b		
Ceyx c. cyanopectus (Lafresnaye, 1840)	bc		
Halcyon chloris collaris (Scopoli, 1786)	b		
Halcyon smyrnensis gularis (Kuhl, 1820)	c	e	
Halcyon coromanda major (Temminck and Schlegel, 1848)	e		
Halcyon l. lindsayi (Vigors, 1831)	bc		e
Family Meropidae			
Merops p. philippinus (Linne, 1766)	bcd		
Merops viridis americanus (P.L.S. Muller, 1776)	be		
Family Bucerotidae			
Penelopides panini manilloe (Boddaert, 1783)	bd		
Order Piciformes			
Family Capitonidae			
Megalaima h. haemacephala (P.L.S. Muller, 1776)	b	e	e
Family Picidae			
Dendrocopus maculatus validirostris (Blyth, 1849)	e	e	e
Chrysocolaptes lucidus haematribon (Wagler, 1827)			e
Order Passeriformes			
Family Pittidae			
Pitta e. erythrogaster (Temminck, 1823)	cd		e
Family Alaudidae			
Alauda gulgula wolfei (Hachisuka, 1930)	a		

Table 1 (Continued)

ORDER/ FAMILY/ SCIENTIFIC NAME	Alviola[1] (1977)	Mendoza Custodio and Phan[2] (1986)	Mendoza[3] (1986)
Family Hirundinidae			
Hirundo rustica gutturalis (Scopoli, 1786)	be		
Hirundo tahitica abbotti (Oberholser, 1917)	bd		
Family Campephagidae			
Coracina s. striata (Boddaert, 1783)	e	e	e
Lalage m. melanoleuca (Blyth, 1861)	e		
Lalage nigra chilensis (Meyen, 1834)	e		
Family Dicruridae			
Dicrurus b. balicassius (Linnaeus, 1766)	bcd		
Family Oriolidae			
Oriolus c. chinensis (Linnaeus, 1766)	be		
Family Corvidae			
Corvus macrorhynchus philippinus (Bonaparte, 1853)	be		
Family Paridae			
Parus e. elegans (Lesson 1831)	cd	e	e
Family Sittidae			
Sitta frontalis isarog (Rand and Rabor, 1967)	d		d
Family Rhabdornithidae			
Rhabdornis m. mysticalis (Temminck, 1825)	e	e	e
Family Timaliidae			
Stachyris capitalis affinis (McGregor, 1907)			e
Family Pycnonotidae			
Pycnonotus g. goiavier (Scopoli, 1786)	bd	e	e

Table 1 (Continued)

ORDER/ FAMILY/ SCIENTIFIC NAME	Alviola[1] (1977)	Mendoza Custodio and Phan[2] (1986)	Mendoza[3] (1986)
Pycnonotus u. urostictus (Salvadori, 1870)	ce		e
Hypsipetes p. philippinus (J.P. Forster, 1795)	bcd	e	e
Family Turdidae			
Copsychus l. luzoniensis (Kittlitz, 1832)	cd	e	e
Copsychus saularis deuteronymus (Parkes, 1963)	b		
Saxicola c. caprata (Linnaeus, 1766)	bd		
Monticola solitarius philippensis (P.L.S. Muller, 1776)	e		
Turdus poliocephalus mayonensis (Mearns, 1907)	e		
Family Sylviidae			
Gerygone sulphurea simplex (Cabanis 1872)		e	
Megalurus palustris forbesi (Bang, 1919)	d		
Cisticola exilis semirufa (Cabanis, 1872)	d		
Locustella certhiola ochotensis (Middendorff, 1853)	e		
Phylloscopus olivaceus (Moseley, 1891)			e
Megalurus timoriensis tweeddalei (McGregor, 1908)	d		
Acrocephalus arundinaceus harterti (Salomonsen, 1928)	d		
Orthotomus atrogularis chloronotus (Ogilvie-Grant, 1985)	ce	e	e
Family Muscicapidae			
Rhipidura c. cyaniceps (Cassin, 1855)	acd	e	e
Rhipidura javanica nigritorquis (Vigors, 1831)	cd	e	
Ficedula w. westermanni (Sharpe, 1888)			e

Table 1 (Continued)

ORDER/ FAMILY/ SCIENTIFIC NAME	Alviola[1] (1977)	Mendoza Custodio and Phan[2] (1986)	Mendoza[3] (1986)
Cyornis h. herioti (Ramsay, 1886)	d	e	
Cyornis rufigaster simplex (Blyth, 1870)	e		e
Hypothymis a. azurea (Boddaert, 1783)	cd	e	e
Muscicapa panayensis nigrimentalis (Ogilvie-Grant, 1894)			e
Pachycephala cinerea crissalis (Zimmer, 1918)	c	e	e
Pachycephala p. philippensis (Walden, 1872)	e		
Family Motacillidae			
Motacilla cinerea robusta (Brehm, 1857)	ce	e	e
Anthus novaeseelandiae lugubris (Walden, 1875)	e		
Family Artamidae			
Artamus l. leucorhynchus (Linnaeus, 1776)	b		
Family Laniidae			
Lanius cristatus lucionensis (Linnaeus, 1776)	bcd	e	e
Lanius schach nasutus (Scopoli, 1786)	e		
Family Sturnidae			
Aplonis p. panayensis (Scopoli, 1783)	be	e	e
Acridotheres c. cristatellus (Linnaeus, 1766)	be		
Sarcops c. calvus (Linnaeus, 1766)	be		
Family Nectariniidae			
Anthreptes malacensis birgitae (Salomonsen, 1953)			e
Nectarinia s. sperata (Linnaeus, 1766)	e		e
Nectarinia j. jugularis (Linnaeus, 1766)	d	e	e

Table 1 (Continued)

ORDER/ FAMILY/ SCIENTIFIC NAME	Alviola[1] (1977)	Mendoza Custodio and Phan[2] (1986)	Mendoza[3] (1986)
Aethopyga f. flagrans (Oustalet, 1876)		e*	e*
Aethopyga shelleyi flavipectus (Ogilvie-Grant, 1894)		e	e
Family Dicaeidae			
Dicaeum a. australe (Hermann, 1783)	e	e	
Dicaeum trigonostigma xanthopygium (Tweedale, 1877)	cd		e
Dicaeum p. pygmaeum (Kittlitz, 1833)	e		
Dicaeum hypoleucum obscurum (Ogilvie-Grant, 1894)			e*
Family Zosteropidae			
Zosterops m. meyeni (Bonaparte, 1850)	d	e	e
Zosterops montana whiteheadi (Hartert, 1903)	d		
Family Ploceidae			
Passer montanus saturatus (Stejneger, 1885)	bd	e	
Family Estrildidae			
Lonchura punctulata cabanisi (Sharpe, 1890)	bd		
Lonchura leucogastra everetti (Tweedale, 1877)	bd		
Lonchura malacca jagori (Martens, 1866)	abd		
Padda oryzivora (Linnaeus, 1758)	e		

---

[1] Avifauna of Mt. Makiling. MS Thesis, University of the Philippines, Los Baños, Laguna.

[2] Avifaunal variation from the lakeshore to the forest edge in Los Baños, Laguna. Sylvatrop (In Press).

[3] Optimal time and duration of avian census in a forested area. MS Thesis, University of the Philippines, Los Baños, Laguna.

[a] Original records from literature

[b] University of the Philippines at Los Baños Museum records

[c] Migratory Animals Pathological Survey (MAPS) in Mt. Makiling

[d] Collected by the respective author/s

[e] Observed by the respective author/s

[e*] Observed and collected specimen is available

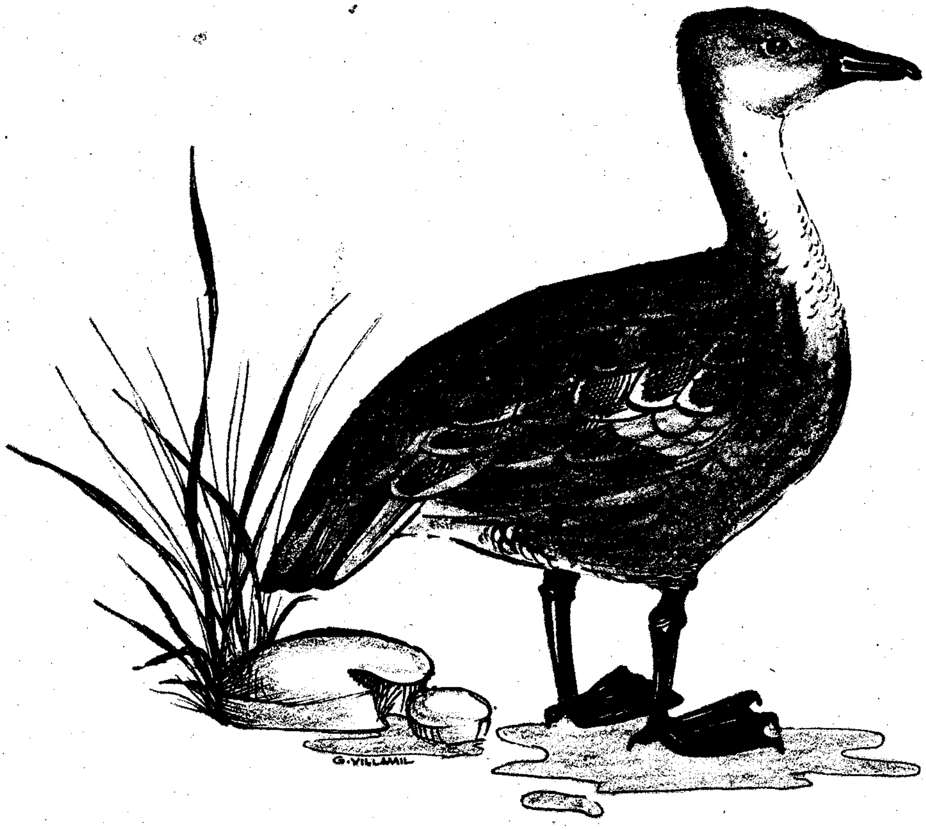

Plate 1. *Dendrocygna arcuata arcuata*
Horsfield, 1824

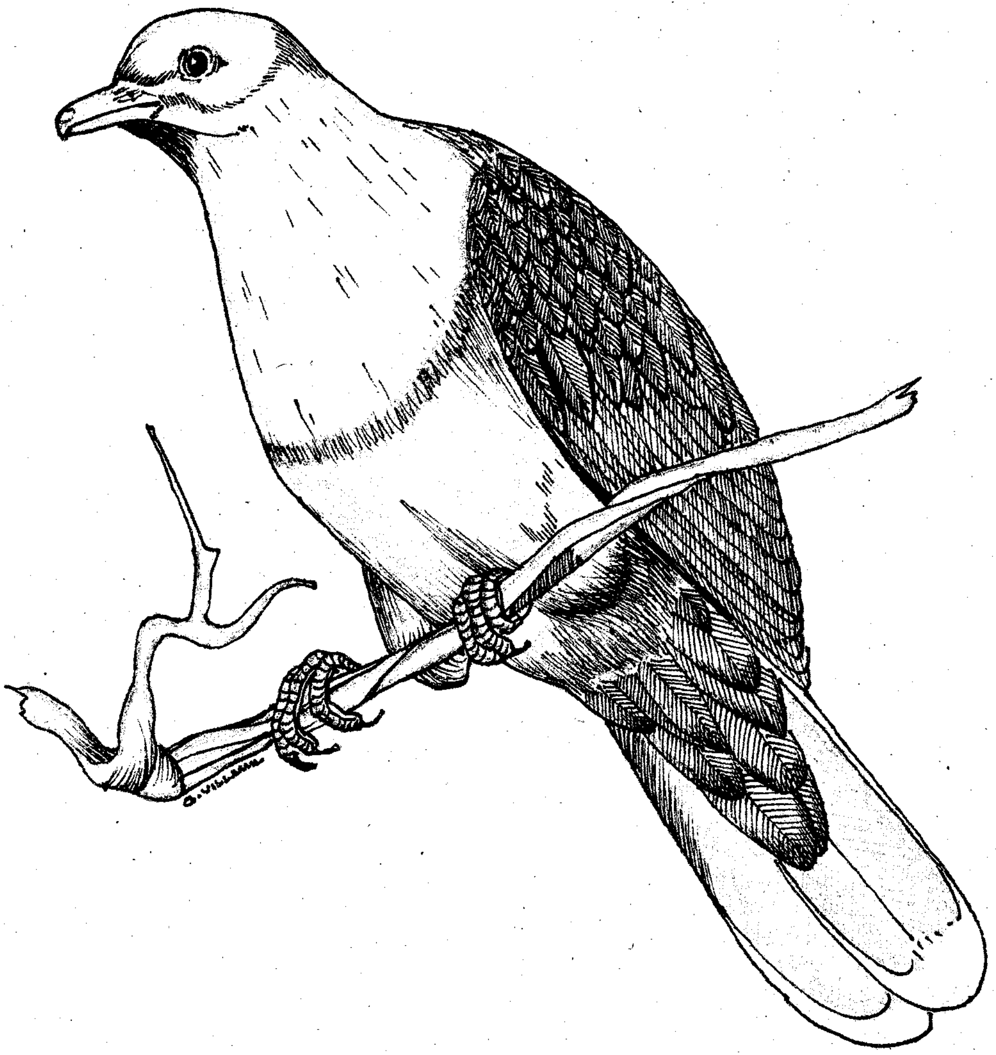

Plate 2. *Ptilinopus leclancheri leclancheri*
Bonaparte, 1855

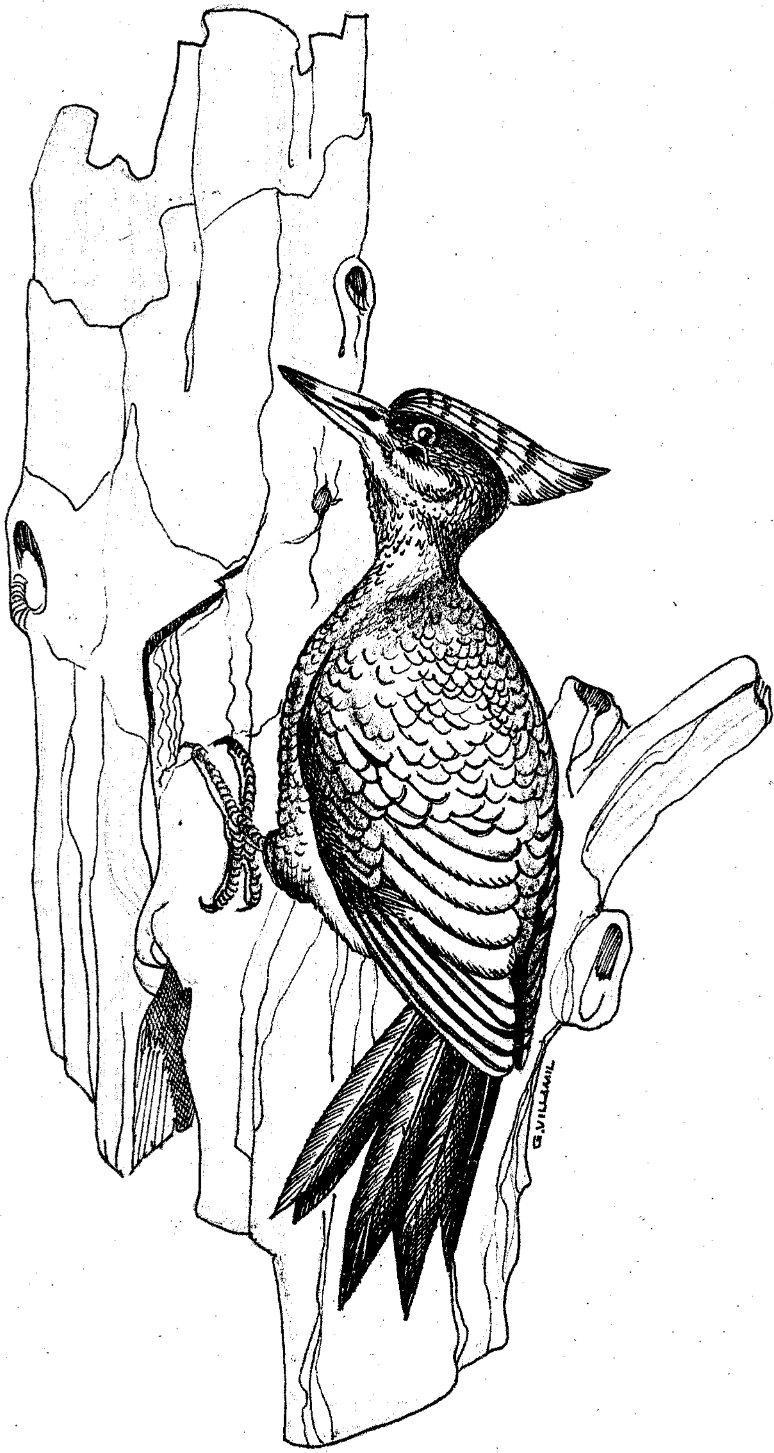

Plate 3. *Chrysocolaptes lucidus haematribon*
Wagler, 1827

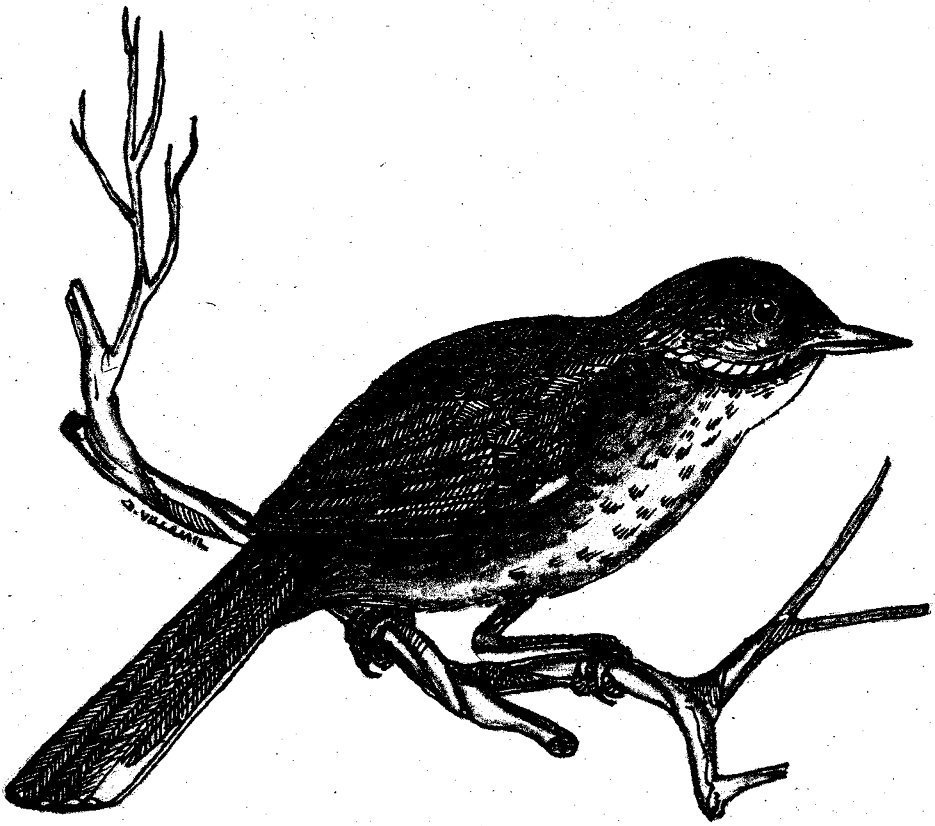

Plate 4. *Stachyris capitalis affinis* McGregor, 1907

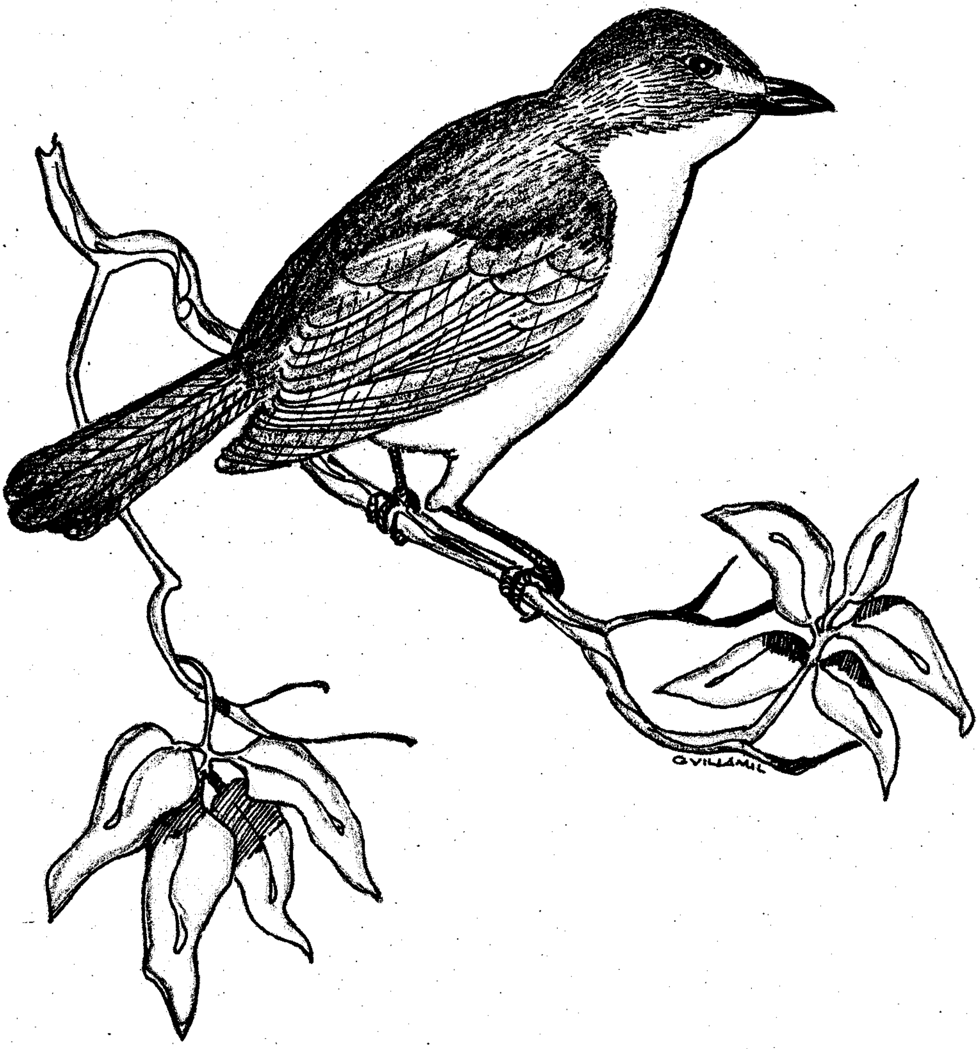

Plate 5. *Gerygone sulphurea simplex*
Cabanis, 1872

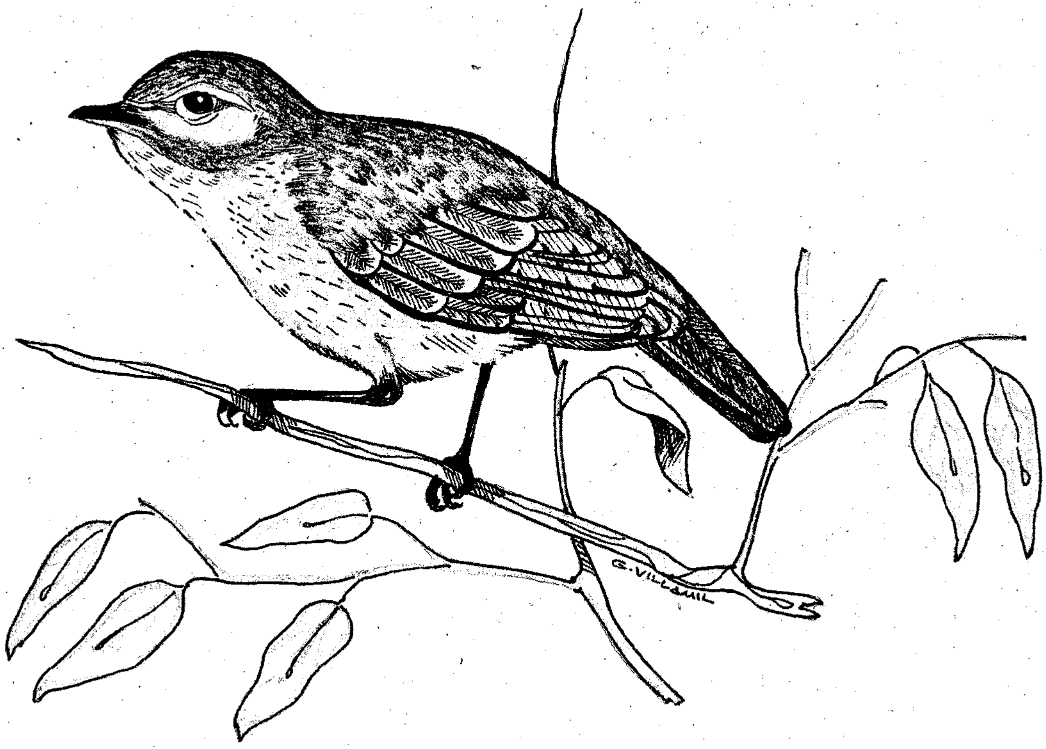

Plate 6. *Phylloscopus olivaceus*
Moseley, 1891

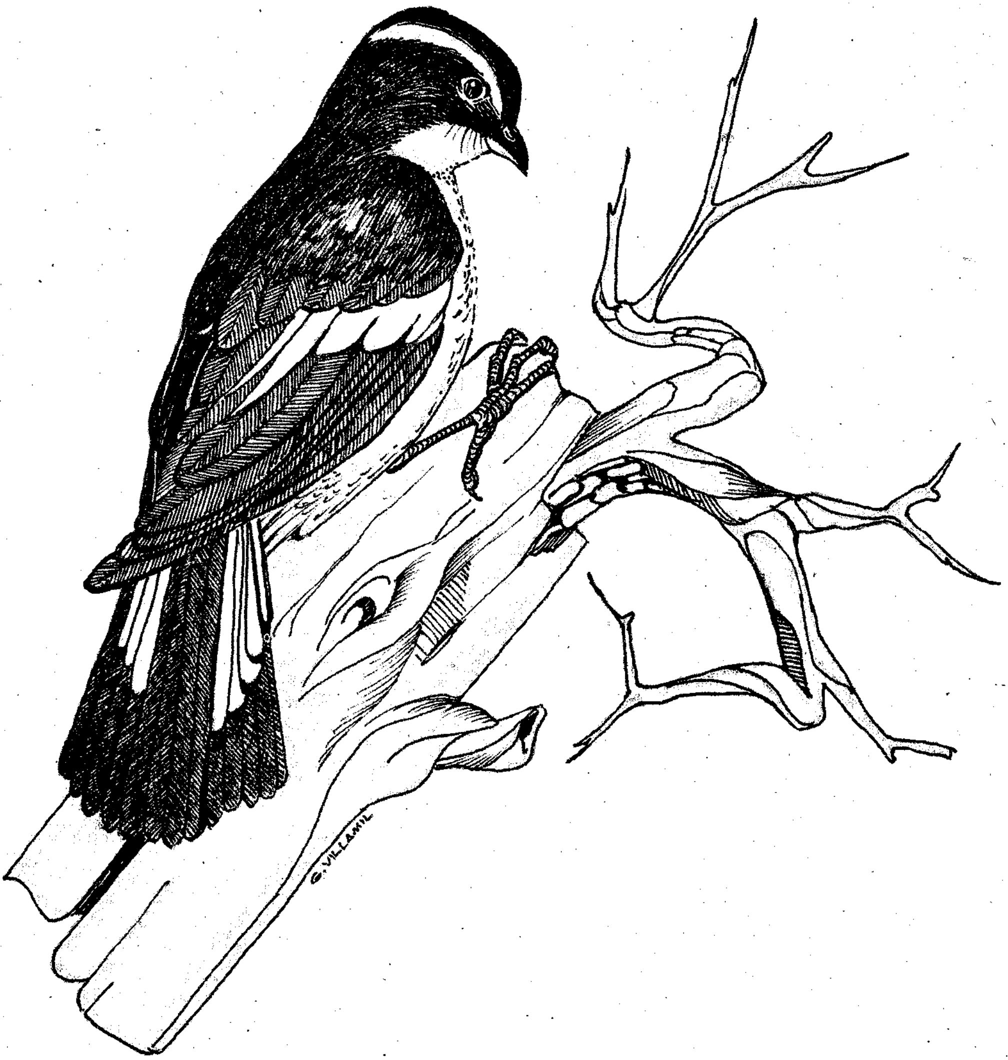

Plate 7. *Ficedula westermanni westermanni*
Sharpe, 1888

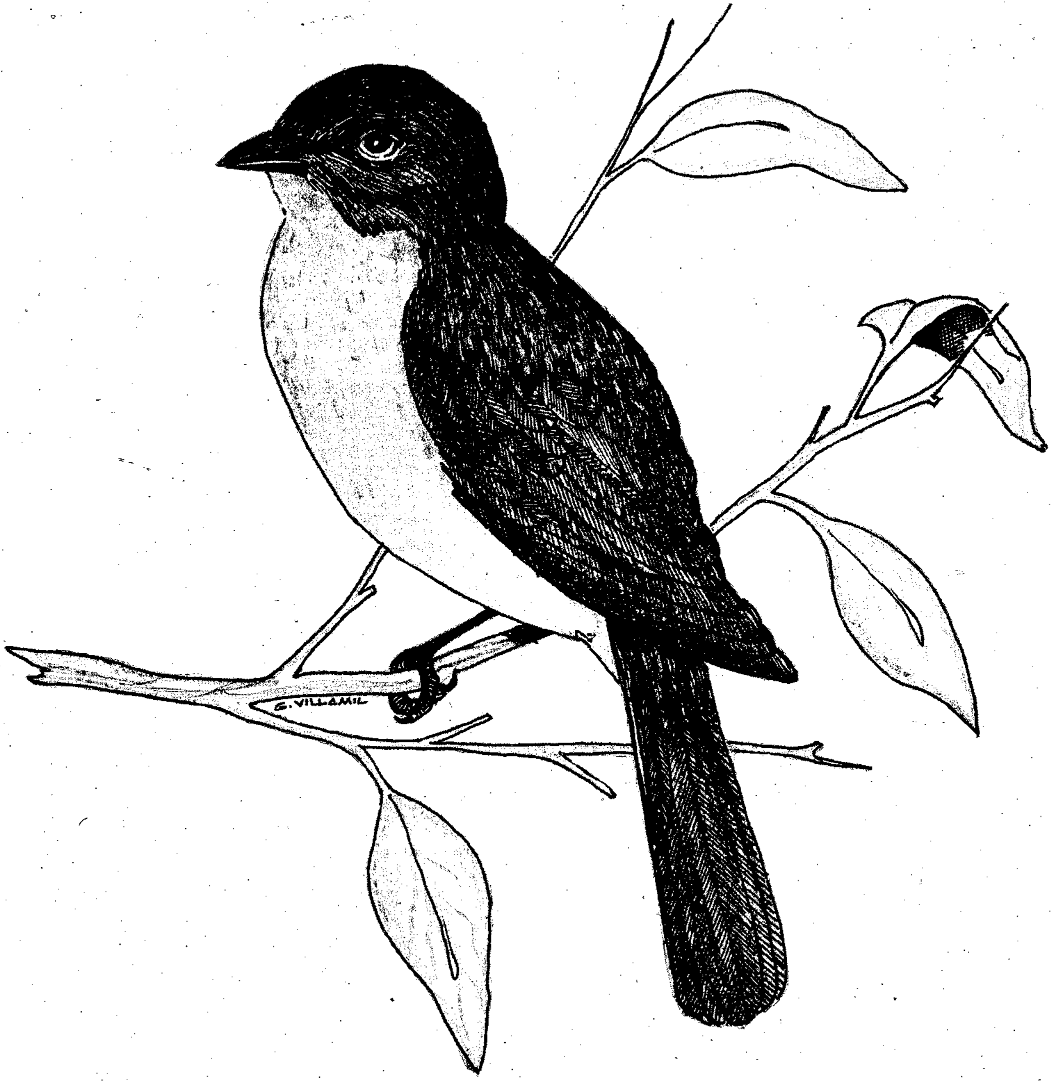

Plate 8. *Muscicapa panayensis nigrimentalis* Ogilvie – Grant, 1894

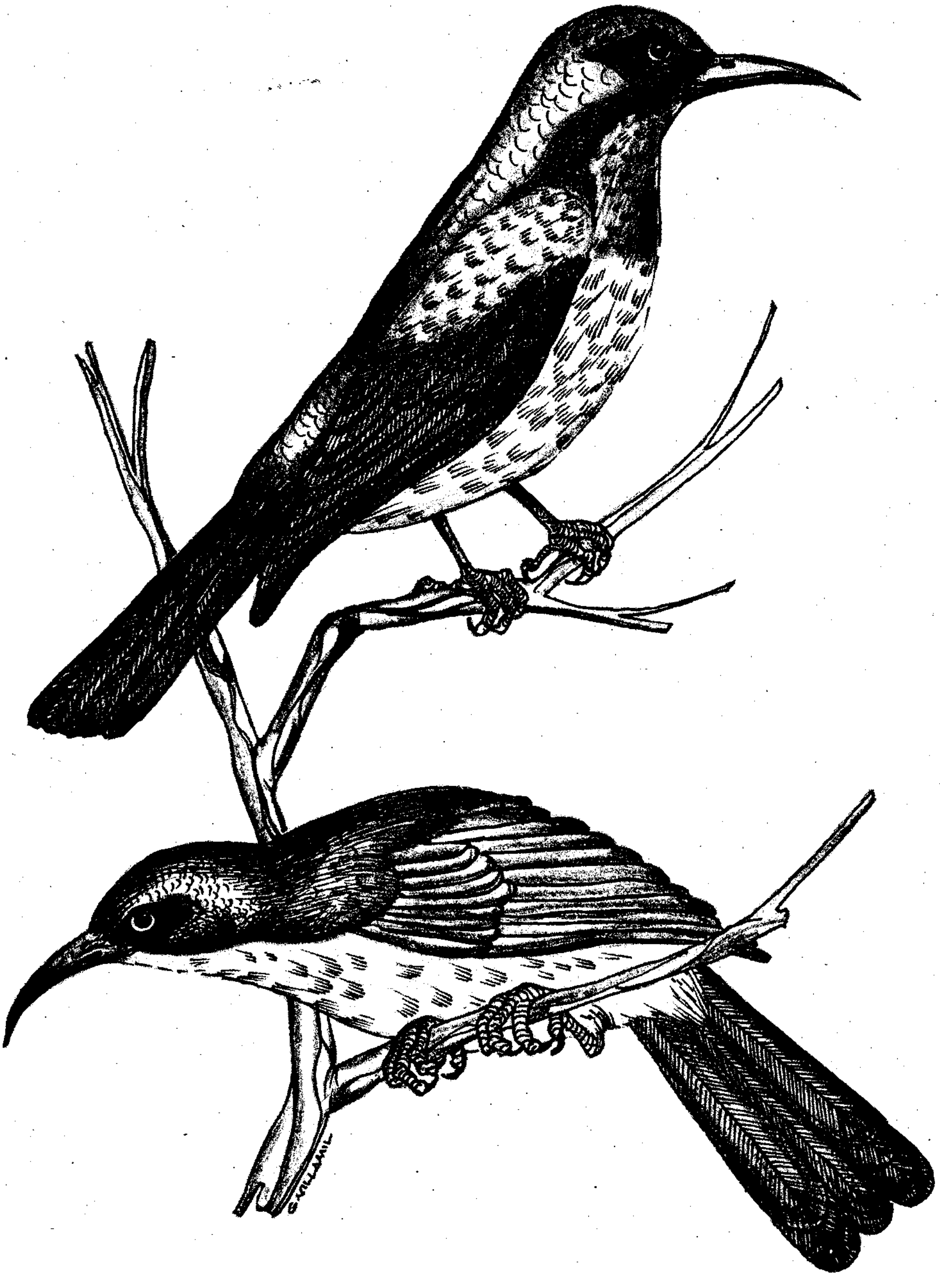

Plate 9. *Anthreptes malacensis birgitae*
Salomonsen, 1953

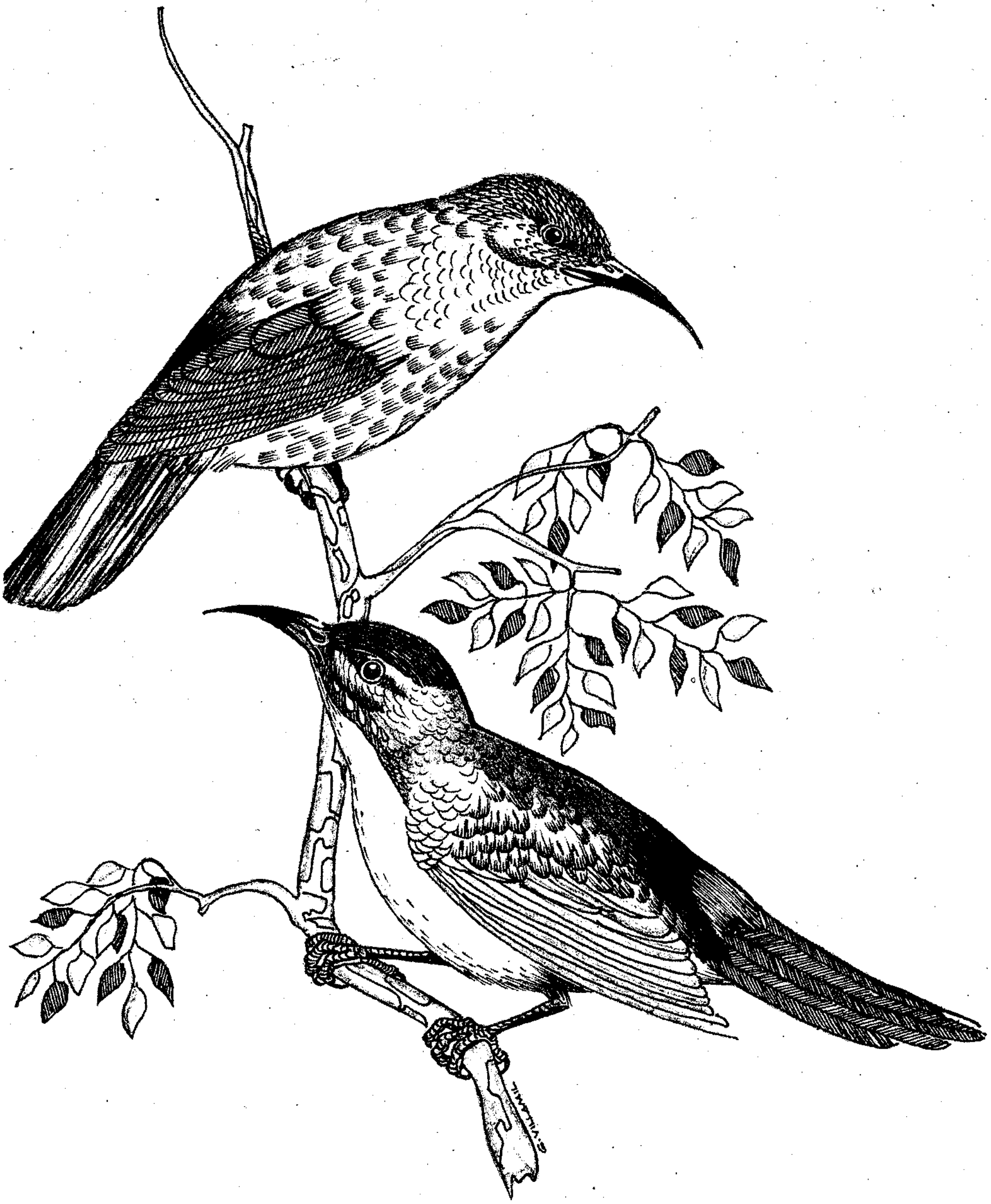

Plate 10. *Aethopyga shelleyi flavipectus*
Ogilvie – Grant. 1894

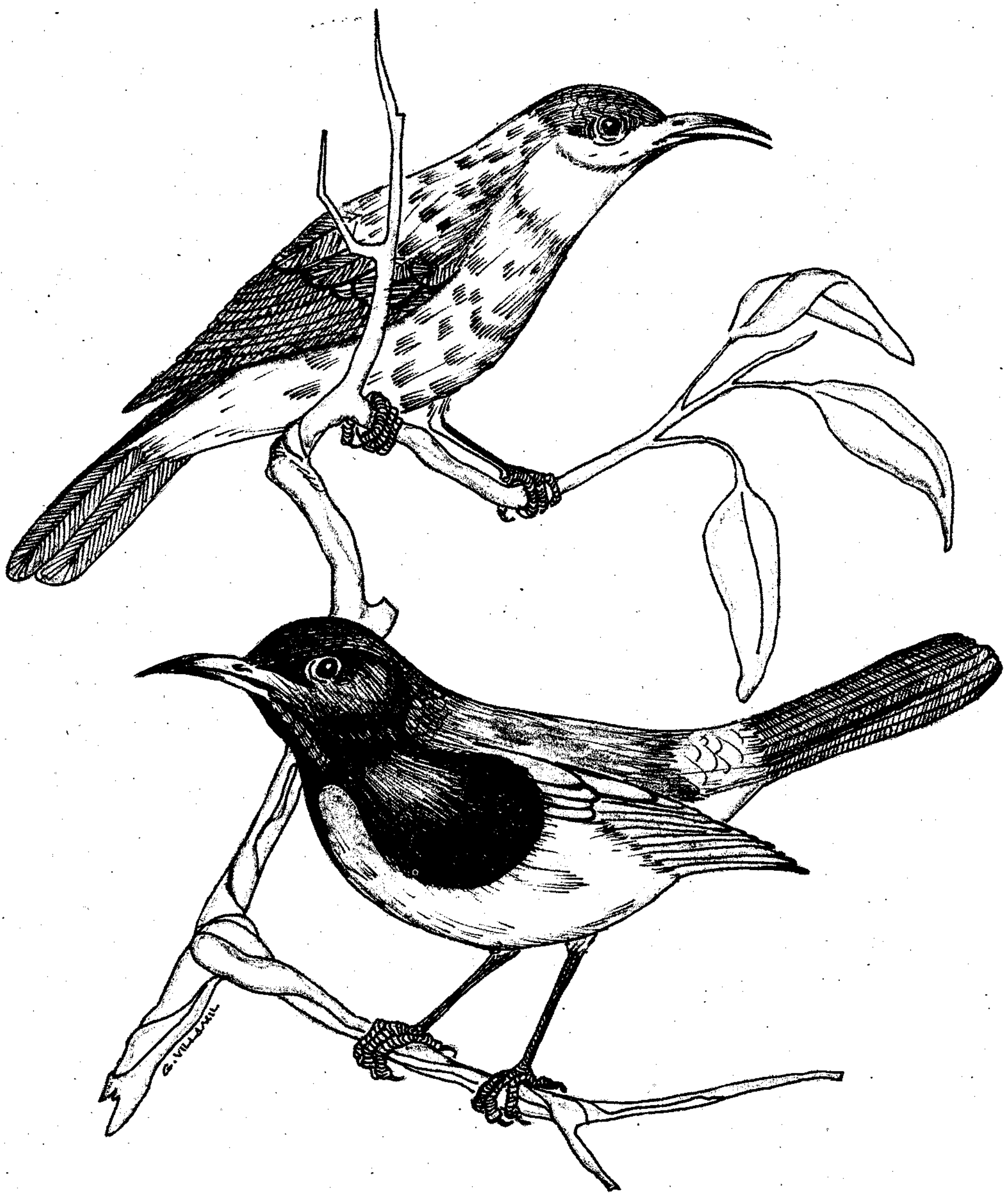

Plate 11. *Aethopyga flagrans flagrans*
Oustalet, 1876

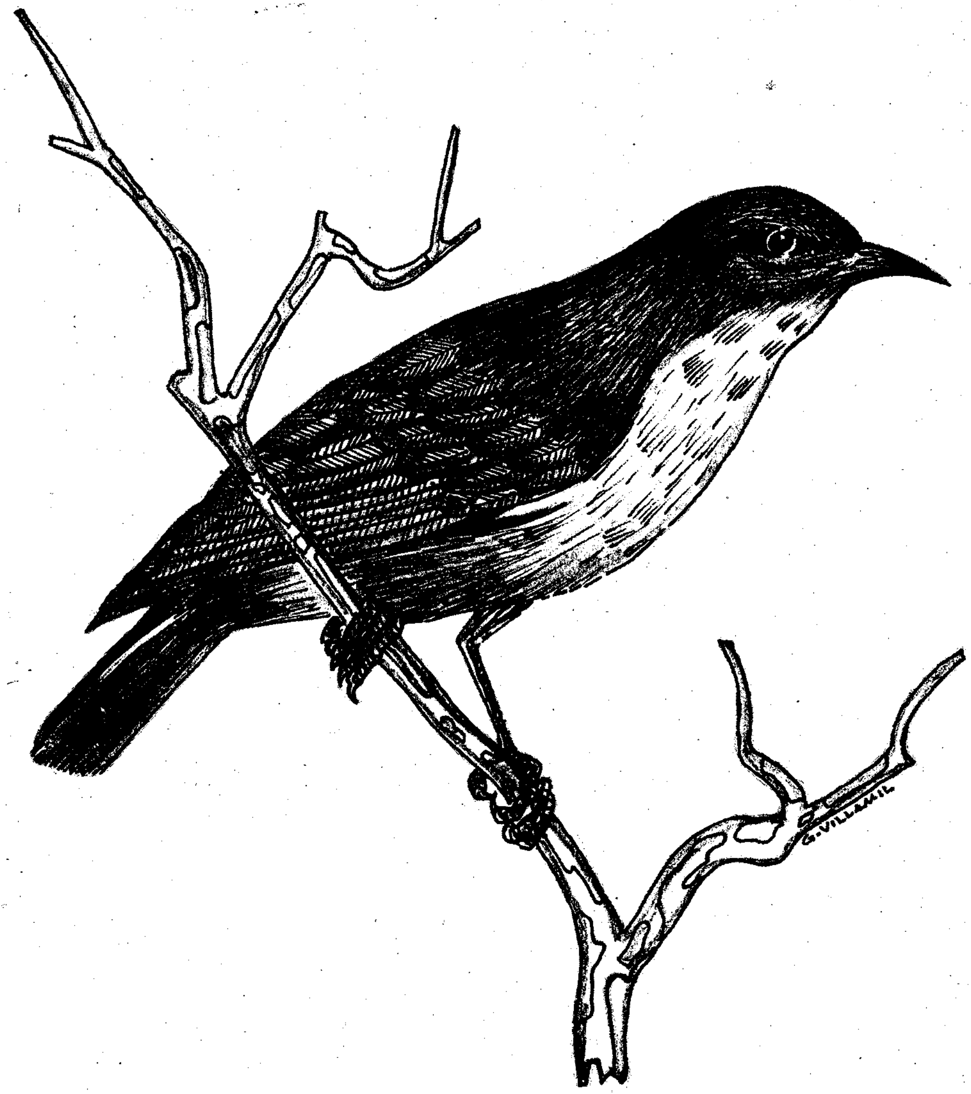

Plate 12. *Dicaeum hypoleucum obscurum*
Ogilvie – Grant, 1894

EXTRACTION AND SEPARATION OF γ – LINOLENIC ACID FROM LIPIDS OF *MORTIERELLA*

CECILE C. MENOR
Chemical Research Development Center
National Institute of Science and Technology
Taguig, Bicutan, Metro Manila

ABSTRACT

The cells of several species of the genus *Mortierella* were grown by fermentation and the fungal lipids were extracted with $CHCl_3/MeOH$ (2:1 v/v) and analyzed for their total, neutral and polar lipids composition by gas liquid chromatography. The main fatty acids of the *M. vinacea* lipids are palmitic 27.30%, stearic 4.80%, oleic 45.40%, linolenic 12.98%, and γ – linolenic fatty acids 7.80%. A concentrate containing 97% of methyl γ – linolenate was prepared from the subsequent urea complex separations of the non-adduct fractions of the fungal lipids.

INTRODUCTION

In Japan, the major sources of fat are imported from other countries. To maintain and preserve these sources, the National Chemical Laboratory for Industry conducts studies on special fungi which could be utilized for industrial purposes.

One important area of interest is the isolation of γ – linolenic acid (all cis 6, 9, 12 octadecatrienoic) which has a growing demand due to its clinical and pharmaceutical uses, as a very active essential fatty acid and the precursor of prostaglandin E_1 and its derivatives (Horrobin, 1982). Previous studies revealed that γ – linolenic acid occurs in significant amounts in various species of the Aceraceae (Bohannon, 1976), Boraginaceae (Kleiman, 1964; Miller, 1968), Ranunculaceae (Spencer, 1970), Scrophulariaceae, Saxifragaceae and Onagraceae families (Wolf, 1983). The most common source is evening primrose *(Oenothera biennis)* seed oil of the Onagraceae family. The presence of this acid in the seed oil of *O. biennis* was first suggested by Heiduschka and Luft (Markley, 1960) and subsequently investigated by Eibner and Riley. The same acid was later reported by Tsuchiya as also present in another species of the same genus, *O. lamarckiana* (Markley, 1960).

Cis 6, 9, 12 Octadecatrienoic acid comprises about 10% of the total fatty acids of the seed oil. Its structure was established by ozonolysis and by isomerization with alkali at 180°C. On bromination, it yields a hexabromostearic acid melting at 196°C and on oxidation with alkaline potassium permanganate, a hexahydrostearic acid melting at 425°C (Markley, 1960).

The purpose of this study is to extract and separate the lipids from the fungal mycelia and to isolate γ – linolenic acid from the fungal lipids of *Mortierella*, particularly *M. vinacea* as a new source of γ – linolenic acid.

MATERIALS AND METHODS

Microorganisms and culture conditions

The strains of the Mortierella species used such as *M. isabellina* IFO 7884, 7824, 8183; *M. vinacea* IFO 8309, 6738; *M. nana* IFO 8794; *M. ramaniana* IFO 8287, 6744 and *M. angulispora* IFO 8187 were obtained from the culture collection of the Institute of Fermentation in Osaka. The fungi were isolated by fermentation and maintained on a yeast extract, malt extract agar (YMA). Glucose was used as the carbon source. Cells were precultured aerobically for 2-3 days at 30°C in 500-mL Erlenmeyer flasks containing 200 mL of growth medium and then 10-mL samples of the culture broth were transferred to 1000-mL Erlenmeyer flasks containing 400 mL of the culture medium. The main culture was grown at 30°C on a glass fermentor of 30-L capacity containing 20 L of culture medium. The nutrient solution compositions of the pre-culture and growth media are tabulated in Table 1.

Extraction of lipids

The fungal cells were separated from the medium by centrifugal filtration. The extraction of lipids was done using a homogenizer in $CHCl_3/MeOH$ 2:1 v/v (Entenman, 1957). Glass beads were added for ease of extraction. Five extractions were done on a sample to obtain the total extractable lipids. The total lipid content was measured gravimetrically. The lipid extracted was stored at 4°C in a $CHCl_3$ solution. The total lipids were fractionated into neutral and polar lipids by elution on a silicilic acid column, initially with $CHCl_3$ and then with MeOH (Vance *et al,* 1967). After evaporation of the eluants, the amount of each lipid was determined gravimetrically. Essentially, all the lipids applied on the column were recovered in two fractions.

Analytical procedures

The lipids were transesterified with 10% BF_3 in methanol according to the method of Metcalf (1967) and analyzed for the fatty acid composition of the total, neutral and polar lipids respectively using Shimadzu gas liquid chromatograph model GC-4CPF equipped with a flame ionization detector and a dual column system of stainless steel column (200 x 0.3 cm) packed with 20% DEGS on chromosorb W AW. Peaks were identified by comparing their retention time with those of authentic standards and quantified by an integrator.

The fatty acid esters were fractionated by the urea complex separation method of Parker and Swern (1957). A weighed amount of urea was dissolved in an equivalent volume of methanol and added to the fatty acid methyl esters. The mixture was warmed to reflux until a clear solution was obtained and allowed to cool at room temperature.

The urea adduct was removed by filtration on a Buchner funnel and then washed with anhydrous diethyl ether. The ether fractions were collected separately from the methanol filtrate and treated with water containing a small amount of HCl. The esters were recovered by extraction with anhydrous ethyl ether. The ether solutions were washed with water until neutral and dried over sodium sulfate. The solvent was removed by flash evaporation under vacuum (Parker, 1957).

Concentration of the γ – linolenic acid was limited to the extracted oil from *M. vinacea.* Several urea complex separations were done on the non-adduct fractions of the fungal lipid fatty acid methyl esters and the non-adduct fractions of the urea complexed adduct fractions. The urea adducts and the non-adducts were analyzed for their fatty acid compositions using Shimadzu gas liquid chromatography as previously described and characterized by proton and ^{13}C -nuclear magnetic resonance spectroscopy.

Proton magnetic resonance was measured by Hitachi R-40 High Resolution spectrometer operating at 90.0 MHz. The spectra were obtained at 35°C by a Varian FT-80 NMR spectrometer operating at 25.2 MHz at 28°C with 10% sample solution in $CDCl_3$. The isolated product was characterized for its ^{13}C- chemical shifts by the assignment of the signals. The ^{13}C and the proton magnetic resonance spectra of the isolated γ – linolenic acid has been compared with the ^{13}C-MNR spectra of the linolenic and α – linolenic fatty acids measured both in $CDCl_3$. $CDCl_3$ was chosen as the solvent because it is a good solvent for methyl esters and it contains deuterium for locking the spectrometer (Bus, 1976). The delta value (δ) was used to report the chemical shifts where $\delta = 0.00$ from TMS with positive values to low field.

RESULTS AND DISCUSSION

The fungal mycelia of *Mortierella* grew well using glucose as the carbon source and utilizing urea and ammonium sulfate as the nitrogen sources. Table 2 gives the amount of cell and lipid formation in the culture medium and that of γ – linolenic acid in the fungal lipids in some species of *Mortierella.* The cells proliferated on a logarithmic rate wherein the nitrogen source was first accumulated followed by an increase in lipid formation. The nutrient solution was completely consumed after 95 hours incubation time.

Generally, the fermentation with species of *Mortierella* has several advantages over the fermentation of the other fungal genera. They have a high carbohydrate and cell mass concentration, greater than 100 g/L which is not suitable for most microoganisms. They also have a high lipid content and the lipid has a cacao butterlike chemical composition. The mycelia are easy to handle in terms of stirring and filtration; and materials are adequate and of low cost. The lipid content to dry cell weight ranges from 36-40% with approximately 95% neutral lipid and 5% polar lipid components. The cultivation of *M. vinacea* gives a high cell mass concentration of 200 g/L and fairly high dry cell weight of 79.7%. The calculated total intracellular lipid was 29.6% containing 7.8% γ – linolenic acid. The lipid content to dry cell weight was 37.1%.

The lipid extracted from *M. vinacea* was used in the succeeding experiments. The existing gas liquid chromatographic condition using the flame ionization detector and 20% DEGS column on chromosorb W AW with Helium as the carrier gas at a column temperature of 190°C gave a good resolution of the fatty acids. The main fatty acids are: 27.8% palmitic, 4.8% stearic, 45.4% oleic, 12.9% linolenic and 7.8% γ – linolenic fatty acids. The chromatogram showed a remarkable difference in retention time of the γ and α – linolenic acids. γ – linolenic acid has a retention time of 18 ± 0.5 minutes while α – linolenic acid has a longer retention time of 20 ± 0.5 minutes.

The urea complex separation procedure of Parker and Swern (1957) was followed in the fractionation of the esters. A series of urea complexations of the non-adduct fractions exhibited a substantial increase in the γ – linolenic acid yield. Further urea complex separations gave a 97% purity of the product and a 50% recovery of the γ – linolenic acid was obtained from the non-adduct fractions. A fairly high purity of γ – linolenic acid was obtained from five subsequent urea complex separations. The urea adduct fraction gave a parallel increase in yield but less substantial compared to the urea non-adduct components.

The isolated γ – linolenic acid was further analyzed and characterized for its chemical structure by proton magnetic resonance and ^{13}C nuclear magnetic resonance. The proton magnetic resonance spectra of 10% esters of methyl linoleate, methyl γ – linolenate and methyl α – linolenate exhibited differences in their double bond cis position as indicated by the area of integration. The methylene groups adjacent to the double bond of the methyl linoleate showed a broadened partially resolved quarter at $\delta = 2.05$ ppm. The low field line overlaps the alpha methylene triplet at $\gamma = 2.25$ ppm. The methylene group between the two double bonds appears in the spectrum as a partially resolved triplet at $\delta = 2.75$. The proton magnetic resonance gave a discernible resolution for the terminal methyl group of the α – linolenic acid methyl ester.

Further characterization was done using ^{13}C-NMR for more sensitive structural difference determination. High resolution ^{13}C Fourier transform nuclear magnetic resonance is an effective tool for the rapid and direct determination of the cis composition of the isolated γ – linolenic acid. It illustrates the ^{13}C-NMR spectra of 20% esters of methyl linoleate, methyl γ – linolenate and methyl α – linolenate. Results gave differences in the signals and the unsaturated carbon resonances of the cis position of the 9, 12 octadecadienoic, 6, 9, 12 octadecatrienoic and 9, 12, 15 octadecatrienoic acids. The carbon chemical shifts are tabulated in Table 3. Chemical shifts are given as delta values in ppm downfield from the internal TMS ^{13}C signal.

The unsaturated carbon resonances of the cis 9, 12 octadecadienoic acid appeared at $\delta = 130.4$ and 128.1; $\delta = 129.7$, 127.7 and 128.5 for 6, 9, 12 octadecatrienoic acid; $\delta = 130.4$, 128.5 and 128.5 for the 9, 12, 15 octadecatrienoic acid respectively. The chemical shift associated with the trienoic moieties is C_{17}. Delta values of 22.7, 22.6 and 20.7 were obtained for methyl linoleate, methyl γ – linolenate and for methyl α – linolenate. C_8 is the external allylic carbon adjacent to a cis double bond with $\delta = 27.2$, $\delta = 25.7$ and $\delta = 27.4$ for the three esters. The internal allylic carbons adjacent to a cis double bond are carbons 11 and 14 and the observed delta values were $\delta = 25.8$; $\delta = 25.7$; $\delta = 25.8$, 25.9 for C_{11} and $\delta = 27.4$; $\delta = 27.3$; $\delta = 25.8, 25.9$ for C_{14} for the linoleate, γ – linolenate and α – linolenate methyl esters respectively. The carbonyl carbon resonance was observed at $\delta = 174.0$. The delta value for the carbon of the fatty acid methyl ester moiety was 14.10 ppm from the TMS internal reference.

ACKNOWLEDGEMENTS

The author wishes to express her heartfelt appreciation to the Japan International Co-operation Agency (JICA) for the opportunity to be trained in Bioorganic Chemistry, to the National Chemical Laboratory for Industry (NCLI) for facilitating the course through the use of their laboratories, to Dr. Osamu Suzuki and Mr. Toshihiro Yokochi of the NCLI for their guidance and valuable advice on the conduct of the research study, and to the National Institute of Science and Technology for their trust and confidence in allowing her to avail of this training.

REFERENCES

1. Bohannon, M.B., and R. Kleinan. 1979. γ – Linolenic acid in Acer seed oils. *Lipids.* **11**:157.
2. Bus, Jan, Izaak Sies, *et al.* 1976. ^{13}C-NMR of Methyl, Methylene and Carbonyl Carbon Atoms of Methyl Alkenoates and Alkynoates. *Chem. Physies Lipids.* **17**:501.
3. Entenman, C., S.P. Colowick and N.O. Kaplan. 1957. Methods in Enzymology. Volume III, Academic Press New York. 310.
4. Folch, J., M. Lees, J.A. Meath and G.H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**:499.
5. Horrobin, D.F. (ed.) 1982. Clinical Uses of Essential Fatty Acids, Eden Press, Montreal and London.
6. Kleiman, R., F.R. Earle, I.A. Wolf and Q. Jones. 1964. Studies on guarseed oil *(Cymmopsis psoralioides). J. Amer Oil Chem. Soc.* **41**:459.
7. Markley, Klare S. (ed.). 1960. Fatty Acids, Their Chemistry, Properties, Production and Uses. Interscience Publishers Inc. New York. 158.
8. Metcalf, C.D., and A.A. Schmitz. 1967. The rapid preparation of fatty acid esters for gas chromatographic analysis. *Anal. Chem.* **33**:363.
9. Miller, R.W., F.R. Earle and I.A. Wolff. 1968. Search for new seed oils. XV. oils of Boraginaceae. *Lipids.* **3**:43.
10. Parker, W.E., and Daniel Swern. 1957. A note on isolation of concentrates of linolenic acid and ethyl linolenate from linseed Oil. *J. Amer. Oil. Chem. Soc.* **43**:43.
11. Spencer, G.F., R. Kleiman, F.R. Earle and I.A. Wolff. 1970. Unusual olefinic fatty acids in seeds oil from two genera in the Ranunculaceae. *Lipids.* **5**:277.
12. Vance, D.E., and C.C. Sweeley. 1967. Quantitative determination of the neutral glycosyl ceramides on human blood. *J. Lipids Res.* **8**:621.
13. Wolf, R.B., R. Kleiman and R.E. England. 1983. New sources of γ – linolenic acid. *J. Amer. Oil Chem. Soc.* **60**:1858.

Table 1. **Composition per liter of nutrient solutions used in the fermentation of the fungal mycelia of *M. vinacea*.**

Nutrient Solution	Growth Medium	Preculture Medium
Glucose	200	60
Urea	5.00	–
NH_4SO_4	2.25	3.0
KH_2PO_4	9.00	3.0
$MgSO_4$	1.00	0.3
NaCl	0.30	0.1
Malt Extract	0.60	0.2
Yeast Extract	0.60	0.2
Peptone	0.30	0.1
Minerals (g/mL)	3.00 mL	1.0 mL
a. $FeSO_4.7H_2O$ 10.0		
b. $CaCl_2.2H_2O$ 1.2		
c. $CuSO_4.5H_2O$ 0.2		
d. $ZnSO_4.7H_2O$ 1.0		
e. $MNCl_3.7H_2O$ 1.0		

Table 2. Cell and lipid formation in the culture medium and the amount of γ – linolenic acid in the fungal lipids in some species of *Mortierella.*

Mortierella Species	IFO No.	Car. Conc. Inc. (g/L) a	Time (h) b	DC (g/L) c	TL (g/L) d	TL/DC (%) e	γ – Linolenic Acid Content in TL	g/L of med. f	% in DC
M. isabellina	7884	270	72	103.50	49.40	47.70	4.40	2.0	1.90
M. isabellina	7824	200	72	76.30	34.90	45.80	6.60	2.10	2.80
M. isabellina	8183	165	72	69.40	24.10	34.70	4.20	0.90	1.30
M. isabellina	8308	200	72	73.20	35.40	48.30	3.50	1.10	1.50
M. isabellina	8309	140	57	43.20	15.90	36.70	10.00	1.40	3.20
M. vinacea	6738	200	95	79.70	29.60	37.10	7.80	2.10	2.60
M. nana	8794	200	96	72.50	33.10	45.60	7.50	2.20	3.00
M. ramaniana	8287	200	96	69.80	27.60	39.50	5.80	1.40	2.00
M. ramaniana	6744	100	56	40.40	12.80	31.80	11.20	1.20	3.20
M. angulispora	8187	190	66	77.60	29.30	37.80	6.40	1.70	2.20

a) carbohydrate concentration; b) incubation time; c) dry cell weight; d) total intracellular lipids; e) percentage total lipids over the dry cell weight; f) amount of linolenic acid in g/L of the culture medium.

Table 3. Fatty acid composition (%) of the fungal lipids of *M. vinacea*.

Fatty Acids	LIPIDS		
	Total	Neutral	Polar
C14:0	0.72	0.78	0.70
C15:0	–	0.05	0.18
C16:0	27.30	30.23	21.90
C16:1	1.12	1.35	1.06
C17:1	–	0.02	–
C18:0	4.78	5.50	1.37
C18:1	45.42	46.30	44.30
C18:2	12.90	8.91	17.78
C18:3	7.80	6.44	12.50

Table 4. Carbon chemical shifts (ppm) of methyl linoleate, methyl γ-linolenate, and methyl α-linolenate in $CDCl_3$ using TMS as the internal reference.

Locant of Double Bond	Locant of Carbon Atom									
	9	8	7	6	5	4	3	2	1	OCH_3
9, 12	130.4	27.2	29.7	29.5	29.5	29.5	25.0	34.2	174.0	51.4
6, 9, 12	127.7	25.7	128.5	129.7	27.0 27.3	29.4 29.2	24.7	34.0	174.1	51.4
9, 12, 15	130.4	27.4	29.7	29.3	29.3	29.3	25.1	34.3	174.0	51.2

Locant of Double Bond	Locant of Carbon Atom								
	18	17	16	15	14	13	12	11	10
9, 12	14.1	22.7	31.7	29.5	27.4	130.2	128.1	25.8	128.3
6, 9, 12	14.1	22.6	31.6	29.4 29.2	27.3 27.0	130.6	128.5	25.7	128.1
9, 12, 15	14.2	20.7	132.1	128.5	25.8 25.9	128.1	127.4	25.8 25.9	128.5

CULTURAL STUDIES ON BLUE-GREEN ALGAE III. *NOSTOC MICROSCOPICUM* CARMICHAEL

GALILEO A. EMRALINO and BIENVENIDA R. RODULFO
National Institute of Science and Technology
Manila, Philippines

ABSTRACT

An edible species of blue-green alga collected from a rice field in San Nicolas, Pangasinan was isolated and purified. Studies were conducted on the cultural requirements of the alga such as cultural medium, pH and initial inoculum weight. The results showed highest yields of the alga in tap water followed by Allison's nitrogen-free medium. The most suitable pH of solution was at pH 6.0 – 7.0 and higher yield was obtained with initially larger algal inoculum added to the culture solution. The alga was identified as *Nostoc microscopicum* Carmichael.

INTRODUCTION

Studies on blue-green algae are mostly on those used in nitrogen-fixation and soil fertilization. However, in many Asian countries such as China, Japan, Indonesia and Malaysia, the blue-green algae are also a popular delicacy. In Northern Philippines, some species are used locally as food.

Thus, blue-green algae are not only nitrogen fixers but also food supplements. With the aid of carbon dioxide, as much as 20% protein is produced in the cells which could be utilized for food and animal feed. One such study was reported by Rodulfo (1983) on an edible species of filamentous *Nostoc* collected from Ilocos Norte.

In this study, another edible species of *Nostoc* was collected from San Nicolas, Pangasinan and its cultural requirements were determined.

MATERIALS AND METHODS

The algae were collected in San Nicolas, Pangasinan at the peak of its abundance in the ricefields. The method used for isolation, purification and maintenance was that of Rodulfo and Emralino (1986). A tube culture of the algae was taken to proper authorities at UP, Diliman, Quezon City for species identification.

Studies on cultural requirements:

Selection of suitable culture medium.

Triplicate sets of one liter capacity dextrose bottles containing 500 mL each of (a) Allison's medium (Allison, 1935), (b) NPK (14-14-14) fertilizer in 10 and 15 ppm dilutions (c) tap water and (d) distilled water were prepared. The solutions were autoclaved at 15 lbs pressure for 30 minutes. Equal numbers of approximately the same sizes and weights of *Nostoc* colonies were distributed into each flask. The cultures were placed on a table and illuminated under four 40-watt flourescent lamps eight hours daily. These were stirred continuously using electric aquarium aerators. Growth was determined weekly by aseptically harvesting the algae in sterile filter paper, taking the fresh weight, measuring the average increase in colony size and then returning to the same containers with fresh solutions. Final harvests were made at the end of the fourth week.

Determination of optimum conditions.

Using a similar set-up as the above and sterilized tap water as growth medium, the effect of pH on algal growth was determined in two series of experiments. In the first series, solutions were adjusted to different pH levels of 5.0, 6.0, 7.0 and 8.0; while in the second series, pHs of solutions were 6.5, 6.8, 7.1, 7.4 and 7.7. Algal yields were determined as before.

Effect of initial inoculum weight on algal growth.

The initial numbers of algal colonies added to the different culture solutions were 15, 30, 45, 60 and 75. The initial weights and pHs of the solutions were taken and the weekly and final yields determined.

The average protein content of the algae was analyzed using Kjehldahl Method of Analysis (AOAC, 1975).

RESULTS AND DISCUSSION

Growth of *Nostoc* in the different culture solutions is shown in Table 1. With NPK (14-14-14) fertilizer at 10 ppm and 15 ppm concentration, the algae were light green in color after a week's growth with a noticeable disintegration of the cell walls of the colonies. This was also observed in cultures grown in distilled water. Within ten days, the algae in the NPK fertilizer and in distilled water were colorless. In the case of tap water and Allison's medium, the colonies were firm and maintained their spherical form and dark green color. With tap water, final colony size and weight averaged 5.67 mm and 2.57 g respectively, while in Allison's medium, the colony size and weight averaged 5.31 mm and 1.97 g respectively.

Of the major mineral requirements of blue-green algae determined by early workers such as Pringsheim (1913), Glade, (1913), and Maertens (1914), phosphate, sulfate and magnesium were found to be necessary for growth. The preference for tap water by this particular strain of *Nostoc* implies that its requirement for growth is sustained by the nutrients present in the solution.

In the study on pH requirements of this algal species, yields were significantly higher between pH 6.0 – 7.0 in both the first and second series (Table 2). At pH 6.8 in the first series, percentile increase in weight and size averaged 3,713% and 236.5% respectively, while at pH 7.0 in the second series, the percentage increase in weight and size averaged 2,187% and 289%. From these observations, while it was frequently stated that blue-green algae grow best under slightly alkaline conditions, this species thrive better in slightly acidic to neutral solutions. Such cultural differences may depend on the ionic ratios of the media used (Allison *et al*, 1935, 1937; Walp and Schopbach, 1942; Kratz and Myers 1955; and Fogg, 1956).

The study on the effect of initial inoculum weight determined by the number of algal colonies on the growth of *Nostoc* showed higher increase in total weight yields when the initial weight was greater (Table 3). As much as 4,700% increase in weight was obtained with an initial 0.365 g *Nostoc* per 500 mL. This conforms to what Pratt (1940) has indicated on the influence of size of inoculum to growth of *Chlorella* in fresh culture medium with optimum initial algal weights of 0.5 – 1.0 g of fresh cells per liter culture solution for maximum yields. Pantastico (1976) used initially one gram of *Nostoc* per liter of culture medium for scale-up production of the algae.

The alga identified as *Nostoc microscopicum** Carmichael had an average protein content of 30% on a moisture-free dry weight.

SUMMARY

An edible blue-green alga collected from a rice field in San Nicolas, Pangasinan was isolated, purified and found to have the following characteristics:

1. Of the five different culture solutions tried, best growth obtained was in tap water followed by Allison's nitrogen-free medium.

2. Algal colony size averaged 5.67 mm with 2.57 g fresh weight using tap water, and 5.31 mm and 1.97 g fresh weight using Allison's medium. The colonies were dark green in color, soft and fragile when grown in Allison's solutions but were colorless and firm in tap water.

3. Best growth of *Nostoc* using tap water was between pH 6.0 – 7.0 in two experimental series.

The study on the effect of initial inoculum weight on algal yield showed that an initially greater inoculum in the culture solution produced significantly higher yields.

The alga was identified as *Nostoc microscopicum* Carmichael with an average protein content of 30.0% based on moisture-free dry weight.

**Nostoc microscopic* Carmichael – colonies generally spherical rarely larger than 1 cm in diameter, soft; filaments loosely entangled; trichomes 5-8 microns in diameter. In moist and submerged shallow water, moist soil grown with grasses.

ACKNOWLEDGEMENT

We would like to thank Mr. Ismael Fetalino and Mr. Rustico Macaspac, Research Assistants, NIST for the collection of the algae; and Mrs. Elvira L. Arrogante, Science Research Assistant II, NIST for preparation of the draft and final typing of the manuscript.

REFERENCES

1. Allen, M.B. The cultivation of Myxophycease. 1952. *Archiv. Mikrobiologie.* Bd. **17**: 34-53.
2. Allison, F.E., and S.R. Hoover. 1953. Conditions which favour nitrogen-fixation by a blue-green alga. Trans. 3rd Int. Cong. *Soil Sci.* **1**: 145-147.
3. Allison, F.E., S.R. Hoover and H.J. Morris. 1937. Physiological studies with the nitrogen-fixing alga, *Nostoc muscorum. Bot. Gaz.* **98**: 433-463.
4. Fogg, G.E. 1956. Nitrogen fixation by photosynthetic organisms. *Ann. Rev. Plant Physiol.* **7**: 51-70.
5. Kratz, W.A., and J. Myers. 1955. Nutrition and growth of several blue-green algae. *Am. J. Bot.* **42**: 282-287.
6. Laing, M. 1978. Algae can boost rice field. *Farming Today.* **4**(4):56-57.
7. Pantastico, J.B., and J.L. Gonzales. 1976. Culture and use of *Nostoc commune* as biofertilizer. *Kalikasan; Philipp. J. Biol.* 5(2):221-234.
8. Pratt, Robertson. 1940. Influence of the size of the inoculum, on the growth of *C. vulgaris* in freshly prepared medium. *Am. J. Bot.* **27**: 1-52.
9. Pringsheim, E.G. 1951. Methods for the cultivation of algae. In Manual of Phycology. An introduction to the algae and their biology. 349.
10. Rodulfo, B.R. 1983. A Study of *Nostoc Linckia* (Roth) Bornet locally known as "Tabtaba". M.S. Thesis. G.A.U. Found. Malabon, Metro Manila.
11. Rodulfo, B.R., and G.A. Emralino. 1986. Cultural studies on blue-green algae. II. *Nostoc carneum* Agardh. *Philipp. J. Sci.* **115** (4):335-346.
12. Singh, R.N. Role of Blue-Green Algae in Nitrogen Economy of Indian Agriculture. Ind. Council of Agric. Res. New Delhi. 1-175. Pyralel Sah at the Times of India Press, Bombay, India.
12. Walp, L., and R. Shopback, 1942. Growth. **6**:33. from The Cultivation of Myxophyceae. 1952. *Archiv. Mikrobiologie Bd.* **17**: 34-53.

Table 1. Effect of different culture solutions on growth of *Nostoc* after 30 days propagation.

Culture Solution	Initial*		Final*		Total Increase (%)	
	Colony Size (mm)	Weight (g)	Colony Size (mm)	Weight (g)	Colony Size	Weight
NPK (14-14-14)	2	0.2171	–	–	–	–
NAWASA Water	2	0.7171	7.62	5.5071	281	1,976
Allison's nitrogen-free medium	2	0.2171	6.0	2.9451	200	1,210
Distilled Water	2	0.7171	–	–	–	–

*Average of three determinations

Table 2. Effect of different pH levels on *Nostoc* growth after thirty days cultivation in 500 cc tap water.

pH	Initial Colony Size (mm)	Initial Weight (g)	Final Colony Size (mm)	Final Weight (g)	Increase in (%)	
					Colony Size	Weight
5	2	0.1430	4.58	0.7551	129	428
6	2	0.1430	7.24	2.9200	262	1,942
7	2	0.1430	7.78	3.2700	289	2,187
8	2	0.1430	2.83	0.1525	41.5	6.6
						=
6.5	2	0.0763	6.18	1.82	209	2,285
6.8	2	0.0763	6.73	2.91	263	3,713
7.1	2	0.0763	6.62	2.36	231	2,993
7.4	2	0.0763	5.60	1.48	180	1,839
7.7	2	0.0763	5.31	0.97	164	1,171

Results are average of three determinations

Table 3. Effect of initial inoculum weight of *Nostoc* on final yield after 30 day propagation in the tap water.

No. of algal Colonies	Initial Colony Size (mm)	Final Weight (g)	No. of algal Colonies	Final Colony Size (mm)	Final Weight (g)	Increase (%) Colony Size	Increase (%) Weight
15	2	0.0730	15	8.2	1.5513	310	2,025
30	2	0.1460	30	5.0	3.9556	150	2,609
45	2	0.2190	45	6.8	8.5253	240	3,793
60	2	0.2920	60	7.1	10.9925	255	3,665
75	2	0.3650	75	7.6	17.4779	280	4,688

Results are average of three determinations

THE EFFECT OF MEDIA STERILIZATION AND OF VARYING SOURCES AND CONCENTRATIONS OF SUGAR AND NITROGEN ON ALCOHOL PRODUCTION BY *SACCHAROMYCES CEREVISEAE* STRAINS

SATURNINA C. HALOS, MA. AUSSIELITA L. LIT
and WILMA T. CRUZ
National Science Research Institute
University of the Philippines
Diliman, Quezon City
Philippines, 3004

ABSTRACT

Several locally isolated and one ATCC yeast strains were tested for their ability to produce alcohol in sterilized and unsterilized media, in various sugar sources, in varying sugar concentrations of molasses and in varying levels of nitrogen supplied by urea. Some strains produced alcohol only in sterilized media while other strains produced as much or sometimes higher alcohol when media was unsterilized. Molasses was the best source of sugar for all strains except MG2065 which produced as much alcohol from brown sugar. All strains except MG2064 produced the highest alcohol concentration at 10% sugar concentration while nitrogen from urea supplied at 10 mg/L was optimal for alcohol production in unsterilized media.

INTRODUCTION

The production of ethanol by *Saccharomyces cereviseae* is commercially undertaken by about 22 distilleries in the Philippines . Previously considered a mere adjunct to an integrated sugar mill, the improvement of alcohol fermentation efficiency has not been given much attention and efficiencies may range from 65-98% in the various alcohol plants.

The present government plan to use alcohol as fuel additive has changed research directions towards improving fermentation efficiency. There is considerable interest in the genetic improvement of yeast for alcohol fermentation. This paper reports results of experiments conducted to determine the reaction of yeast strains to various practices in the fermentation plants. These include the use of varying concentrations of molasses depending upon the season of the year, the use of urea at varying concentrations and the general use of unsterilized wort. Also determined is the reaction of a set of yeast strains to various sugar sources since there is a proposal to use brown sugar for alcohol fermentation.

The production of alcohol from molasses by various strains has been reported (Hipolito *et al*, 1961; Ignacio and Alicbusan, 1967; Flor, 1977; Sabiniano, 1979; Colle *et al*, 1982). There are also several studies on other substrates such as coconut sap. The variation in sugar concentrations used in fermentation by the industry is most often based on observation and experience gathered through the years. Valera (1955) claimed that it is essential as well as economical to work at 12° Brix. Prescott and Dunn (1949) advised that a sugar concentration of 10-18% usually gives satisfactory results. Hipolito *et al* (1961) found that alcohol-producing yeast was tolerant up to 25% total reducing sugar in molasses medium. Almonte (1977) recommended the use of molasses at 10° Brix and 15% sugar to produce high yield of alcohol. Presently, there is a preference in the industry towards the use of higher sugar concentrations for fermentation to minimize the amount of slops that causes environmental pollution. Shvests and Slyuserendo (1970) studied the effect of sterilization of molasses to minimize decrease in concentration of fermentable matter for yeast. They recommend that molasses must be sterilized at 120°C for ten minutes or at 140°C for 5 minutes. However, the reaction of specific strains to sterilization, varying sugar sources and concentrations and varying N-concentration is not yet reported.

MATERIALS AND METHODS

The MG series of yeast strains were locally isolated from fermenting nipa sap and identified as *Saccharomyces cereviseae* by Ms. Lourdes M. Tapay. The strain ATCC 2345 used as control was obtained from the American Type Culture Collection through Dr. Ernesto J. del Rosario.

All strains were streaked and allowed to grow on MYGP slants. After sufficient growth, each isolate was transferred aseptically to sterile molasses medium containing 5% sugar for inoculum build-up and incubated for 24 hours with shaking. Using ten percent (10%) of the inoculum, the fermentation medium (15% sugar) was incubated for 8 hours on a rotary shaker and then allowed to stand at room temperature for 15 hours. Treatment was varied as follows:

1. Sugar sources – Molasses, brown sugar, white sugar and glucose-sucrose mix were used. Sugar concentration was 15%.
2. Sugar concentration – Molasses was used as source of sugar at 15, 20 and 25% sugar concentration.
3. Nitrogen levels – Nitrogen levels used were 10, 20, 30 and 40 mg/L. The recommended amount of 20 mg/L solution served as control.

Each treatment was replicated three times in 100 mL fermentation media with initial pH of 5.0.

Percent alcohol was determined using an ebulliometer and total sugar using the phenol-sulfuric assay method.

Statistical tests were carried out by CRD two factorial.

RESULTS AND DISCUSSION

1. *Effect of media sterilization*

Sterilization of media caused a very dramatic increase in alcohol production by the majority of yeast strains tested (Table 1). MG2S13, MG2S15, MG2S16 and MG2S19 showed marked reduction in alcohol production in unsterilized molasses medium compared with sterilized molasses medium. This probably indicates that these strains are more sensitive to the presence of contaminants in molasses medium or it could also be due to the inability of the strains to utilize sucrose. Sterilization inverts the sucrose into glucose and fructose specially at low pH. Hence, either Strains MG2S14, MG2S18, MG2S20, MG2S21 and MG2S35 are more resistant to contamination or they possess more active invertases since their alcohol production in sterilized as well as unsterilized media were comparable. Strains MG2S14 produced higher alcohol yield in unsterilized medium implying that it is most resistant to contamination or it possesses the most active invertase system.

2. *Effect of sugar source and sterilization on alcohol production*

The alcohol production by five yeast strains, MG 2063, MG 2064, MG 2065, MG 2A33 and ATCC 2345 is presented in Table 2. Statistical analysis of the data indicated significant differences among the five strains and therefore their performance is very much affected by the sugar source and sterilization. All strains produced alcohol best in molasses medium whether sterilized or not. The highest alcohol concentration was produced by MG 2064. MG 2065 produced the same amounts of alcohol in sterile and unsterile molasses medium. But the other three strains produced slightly higher amounts of alcohol in sterilized than in unsterilized molasses medium. Brown sugar was the next best source of sugar to molasses. The higher amounts of alcohol produced in molasses compared with the other sugar sources indicates the positive effect on alcohol fermentation of organic substances such as vitamins, amino acids and other sugars and mineral salts found in molasses. Such substances could also be found in brown sugar but in lesser amounts which explains why brown sugar was the second best source. MG 2065 produced as much alcohol in unsterilized brown sugar medium as in sterile and unsterile molasses medium indicating its utilization of additional heat-sensitive substances in the brown sugar medium. Alcohol production in unsterilized white sugar and unsterilized glucose-sucrose mix was comparable in all strains. This is expected since these fermentation media have not been supplemented with mineral salts necessary even for normal growth, the only supplement being urea as N-source. The least amount of alcohol was produced by all strains in sterilized white sugar.

The production of slightly more alcohol in sterilized molasses medium compared with unsterilized molasses medium could be due to the presence of more contaminants in unsterilized medium. The possible effect of sucrose inversion to glucose and fructose could not explain this difference because in white sugar and glucose-sucrose mix media, the reverse was observed. More alcohol was produced in these unsterilized medium, probably because in these media, alcohol production was affected by loss of trace organic nutrients due to heating during sterilization.

3. *Effect of varying sugar concentrations and sterilization on alcohol production*

As expected, the production of alcohol increased with increasing sugar concentrations between 15 and 25% (Table 3) but only in sterilized medium. In unsterilized medium, the tendency for all strains except Strain MG 2064 was a decrease in alcohol production when the sugar concentration was increased from 15 to 25%. This could be due to the higher population of contaminants since in media with higher sugar concentration, more molasses was added. Strain MG 2064 was probably quite resistant to contamination; its alcohol production in sterile as well as unsterilized media was comparable in media with 15% and 20% sugar. However, at 25% sugar concentration, MG 2064 produced lower alcohol in unsterilized medium than in sterilized medium since there was probably a higher initial contaminant population.

4. *Effect of varying levels of nitrogen on alcohol production*

The yeast strains' reaction to increasing levels of urea from 10 mg-40 mg/L was a decrease in alcohol production (Table 4). All strains produced their highest alcohol yields at 10 mg/L in unsterilized medium but Strain ATCC 2345 produced comparable levels in unsterilized medium supplemented with 20 mg urea and Strain MG 2064 in sterilized medium with 20 mg urea. In this set of experiments, the difference in alcohol produced due to the use of sterile and unsterile media was more apparent with a general trend for all strains to produce higher alcohol amounts in unsterilized media. This is especially true when the concentration of urea used was 10 mg/L. These results show that urea at 10 mg/L in unsterilized wort was most favorable for alcohol fermentation by all five strains tested.

SUMMARY AND CONCLUSIONS

1. The ability to produce high amounts of alcohol in sterile or unsterile molasses medium is strain-specific.

2. Molasses was the best source of sugar for all strains tested, except Strain MG 2065 which produced comparable alcohol amounts in both sterilized and unsterilized molasses and unsterilized brown sugar.

3. The optimum sugar concentration for alcohol production using unsterilized wort in all strain was 15%, except Strain MG 2064 which produced higher alcohol at 20% sugar.

4. Urea of 10 mg/L was found to be optimal for alcohol production in all strains.

ACKNOWLEDGEMENT

This study was funded by the Ferdinand E. Marcos Foundation through the National Institutes of Biotechnology and Applied Microbiology (BIOTECH).

REFERENCES

1. Almonte, E.R. 1977. Production of sugar cane wine (Basi) from molasses. BSFT. Unpublished thesis. University of the Philippines at Los Baños.
2. Colle, B.D., J.C.L. Garcia and E.J. del Rosario. 1982. Evaluation of several yeast isolates and *Zymomonas* strains for alcohol production. *Proceedings of the 29th PHILSUTECH Convention,* Cebu City. Aug. 4-6, 1983. pp. 188-202.
3. Flor, P.Q. 1977. Ethanol production of four yeasts in different molasses sources. Undergraduate Thesis. UPLB. Unpublished.
4. Hipolito, D.G., M.A. Palo and V.S. Quisumbing. 1961. A comparative study of six local and one foreign strains of yeast in the production of ethyl alcohol from black-strap molasses. *Philipp. J. Sci.* **90**(2): 249-257.
5. Ignacio, L.C., and R.V. Alicbusan. 1966. A comparative study of the alcohol fermentation by three yeast isolates using molasses as the raw materials. *Philipp. Phytopathol.* **3**:55-59.
6. Prescott, S.C., and C.G. Dunn. 1959. Industrial Microbiology. New York: McGraw-hill Book Company, Inc. 2nd ed. pp. 116-117.
7. Sabiniano, N.S. 1979. Screening of yeast isolates for high alcohol production in molasses medium. Undergraduate Thesis. UPLB. Unpublished.
8. Shvests, V.N., and T.P. Slyusarendo. Alteration of the technological characteristics of molasses during heat treatment. *Sugar Industry Abstract.* 71743:86.
9. Valera, L.V. 1955. Treatment of mash in the fermentation of final molasses. Proceedings of the Philippine Sugar Technologists.

Table 1. Alcohol production by some yeast strains in sterlized and unsterilized molasses media containing 15% sugar and 20 mg urea/L at pH 5*.

Yeast	AMOUNT OF ALCOHOL PRODUCED AFTER 24 HOURS INCUBATION (% v/v)	
	Sterilized	Unsterilized
MG-2S-13	2.29	0.03
-14	4.25	7.62
-15	7.48	1.39
-16	4.13	0.00
-18	2.33	1.60
-19	6.33	0.00
-20	3.09	2.60
-21	1.75	2.44
MG-2S-35	8.88	7.72

*Each value represents the average of three replicates.

Table 2. Alcohol production (% v/v) * by five yeast strains in sterilized and unsterilized wort with initial pH 5 and supplemented with various sugar sources equalized at 15% total sugars and 20 mg urea/L after 24 h fermentation.

Strain	Sugar source **							
	Molasses		White sugar		Brown sugar		Glucose-sucrose mix	
	SM	UM	SM	UM	SM	UM	SM	UM
MG-20-63	6.17^{abcde}	5.17^{bcdef}	0.22^{k}	3.55^{fghij}	4.48^{defgh}	4.59^{cdefgh}	2.49^{ij}	3.15^{ghij}
MG-20-64	7.43^{a}	7.05^{a}	0.55^{k}	3.58^{fghij}	3.89^{fghij}	4.59^{cdefgh}	3.67^{fghi}	3.50^{fghij}
MG-20-65	5.88^{abcde}	5.96^{abcde}	0.08^{k}	3.58^{fghij}	3.85^{fghij}	6.40^{abc}	2.44^{ij}	3.63^{fghij}
MG-2A-33	6.53^{ab}	4.75^{bcdefg}	0.38^{k}	4.26^{efghi}	4.59^{cdefgh}	$4.98^{bcdefgh}$	2.21^{ij}	3.82^{fghij}
ATCC 2345	6.24^{abcd}	5.05^{bcdefg}	0.38^{k}	3.74^{fghi}	3.41^{fghij}	$4.89^{bcdefgh}$	3.01^{ghij}	3.74^{fghij}

*Each value is the average of 3 replicates

**Means followed by the same letter are not significantly different from each other at 5% using DMRT

SM – Sterilized media

UM – Unsterilized media

Table 3. **Alcohol production (% v/v)* of five yeast strains using different sugar concentrations supplemented with 20 mg/L urea at initial pH 5.0 in sterilized and unsterilized molasses media at 24 h fermentation.**

Isolate	Sugar concentration (% Total sugar**)					
	15		20		25	
	SM	UM	SM	UM	SM	UM
MG-2O-63	6.48^{fe}	6.69^{de}	6.79^{de}	5.78^{ghi}	8.44^{b}	4.26^{*}
MG-2O-64	6.69^{de}	6.37^{efg}	7.56^{c}	7.29^{cd}	9.65^{a}	7.24^{cd}
MG-2O-69	6.61^{de}	6.84^{de}	7.24^{cd}	5.26^{hijk}	8.83^{b}	4.32^{e}
MG-2A-33	6.65^{de}	6.32^{efg}	6.69^{de}	5.92^{fgh}	9.48^{a}	5.65^{ghi}
ATCC 2345	4.88^{kl}	6.52^{fe}	5.88^{fgh}	6.33^{efg}	6.60^{de}	5.26^{hijk}

*Each value is the average of 3 replicates

**Means followed by the same letters are not significantly diferent from each other at 5% using DMRT

SM – Sterilized media

UM – Unsterilized media

Table 4. **Alcohol production (% v/v) * by yeast strains in sterilized and unsterilized molasses media supplemented with urea after 24 h fermentation.**

Isolate	Nitrogen concentration (mg urea/L)** 10		20		30		40	
	SM	UM	SM	UM	SM	UM	SM	UM
MG-2O-63	3.50^{jklm}	6.16^{abc}	4.21^{ghijk}	5.73	3.23^{jklmno}	4.44^{fghij}	2.97^{lmnop}	3.93^{ijklm}
MG-2O-64	4.88^{efghi}	7.14^{a}	6.06^{abcd}	5.40^{cdefg}	2.60^{nop}	4.95^{defghi}	0.06^{q}	2.68^{nop}
MG-2O-69	4.79^{efghi}	6.15^{abc}	4.25^{ghijk}	4.82^{efghi}	3.16^{klmno}	2.88^{lmnop}	3.05^{klmno}	2.11^{op}
MG-2A-33	2.69^{nop}	6.80^{ab}	4.15^{hijkl}	5.30^{cdefgh}	3.34^{jklmno}	4.42^{fghij}	3.32^{jklmno}	3.12^{klmno}
ATCC 2345	2.55^{nop}	6.84^{ab}	3.74^{ijklmn}	6.10^{abcd}	2.15^{op}	5.60^{cdef}	1.81^{p}	4.08^{ijklm}

*Each value is the average of 3 replicates

**Means followed by the same letters are not significantly different from each other at 5% using DMRT

SM – Sterilized media

UM – Unsterilized media

LIFE HISTORY AND CONTROL OF BEE MITE *VARROA JACOBSONI* OUDEMANS

CLEOFAS R. CERVANCIA and AURORA C. ASPIRAS
Institute of Biological Sciences
University of the Philippines at
Los Baños, College, Laguna

ABSTRACT

The biology and control of bee mite, *Varroa jacobsoni* Oudemans were studied. The life cycle occurs primarily in the bee cell. The female mites attach themselves to the emerging adult bees while the males stay inside the cell.

Tetradifon strips, naphthalene and leaves of plant species such as *Premna odorata* Blanco ("alagaw"), *Blumea balsamifera* (L.) DC, ("sambong"), *Gliricidia sepium* (Jacq) Steud, ("madre de cacao") and *Hyptis suaveolens* Poir, ("suob kabayo") were tested for the control of bee mites.

High mortalities were observed in naphthalene and Tetradifon-treated colonies and among the plant materials evaluated, *H. suaveolens* and *G. sepium.*

INTRODUCTION

The bee mite, *Varroa jacobsoni* Oudemans has been the foremost problem of the world beekeeping community (De Jong *et al,* 1982). The mites directly feed on the larvae and pupae, thus killing and adversely affecting the developing bees. When the bee emerges, the wings and appendages are deformed. Infested colonies weaken and eventually die, thus reducing honey production.

V. jacobsoni was first described by A.D. Oudemans from specimens collected in brood cells of *Apis mellifera* in Java (Pandey, 1967). Delfinado (1963) reported its occurrence in the Philippines.

The life history of *V. jacobsoni* was studied in Japan on artificial medium (Sakai *et al,* 1979). Other reports on the biology of bee mites were those of Sevilla (1965), Akratanakul (1975) and Choi and Woo (1973).

Several studies have been reported on the control of *V. jacobsoni.* Pandey (1967) observed that the removal of dead brood and queen minimized population build up of mites. Chlorobenzilate strips smouldered in the hives have been widely used (Coleman, 1962; Laigo and Morse, 1968; Reminghoff and Miller, 1984).

De Jong *et al* (1982) made a comprehensive literature review on *V. jacobsoni* and other mite pests of honeybees.

This study aimed to determine the life cycle, habits, distribution and control of *V. jacobsoni*. The experiments were done in the Institute of Biological Sciences, U.P. at Los Baños; Sariaya, Quezon and selected apiaries in Laguna, Bulacan, Baguio, Cavite, Pangasinan and Metro Manila, from August, 1984 to November, 1985.

MATERIALS AND METHODS

Life History Studies

Three colonies in three frames were obtained from an established apiary. These colonies were not treated with acaricides to produce a natural infestation of *V. jacobsoni* and thus a constant source of mites for the study. They provided an adequate supply of worker bees and pupae which served as hosts of the mites.

When natural bee mite infestation occurred, the developing pupae were taken out from their cell with a pair of forceps and placed in Petri dishes. Paired male and female mites were picked up with a fine camel's hair brush and transferred to a test tube (6 x 3 cm) containing healthy pupa and plugged with a wad of cotton. The pupa was changed whenever necessary. Other sets of adult mites were introduced to adult bees confined in Petri dishes. The mites were observed at least two times a day for oviposition and duration of each stage. However, due to high mite mortality in the laboratory, the development of mites was directly observed from the brood cells.

Toxicity Studies

The test acaricidal materials used were Tetradifon strips, naphthalene and plant species such as *Premna odorata* or "alagaw", *Blumea balsamifera* or "sambong", *Gliricidia sepium* or "madre de cacao" and *Hyptis suaveolens* or "suob kabayo".

Ten grams each of the ground leaves of test plants and three grams pulverized naphthalene were evenly spread on individual wooden frames (45 x 34 x 1.5 cm) covered with 8-mesh wire. Three frames were placed under the brood nest and on the bottom board of test colonies. The Tetradifon strip (5 x 2.5 cm) was smouldered in other colonies. The entrance of the hive was closed for 30 minutes during the treatment. A piece of white paper (45 x 34 x 1.5 cm) was placed at the bottom of each treated colony to facilitate counting of dead mites. Moribund mites were considered dead. All treatments with three replications were done at one day intervals. Mortality counts were taken 24 hours after treatment. Arc sine transformation was resorted to because of the wide range of percentage mortality obtained from the treatments. All data were analyzed using Completely Randomized Design.

The initial mite populations were estimated by slicing three 3.8 x 5 cm brood cells per colony. Adult bees were brushed to sample the mites adhering to their bodies, while the brood cells were examined for the number of mites present. From these counts, the total population per colony was extrapolated.

All toxicity tests were done in Mr. Angel Velasco's bee farm in Sariaya, Quezon.

Distribution of V. jacobsoni

Mite species were surveyed in selected apiaries in Metro Manila, Bulacan, Cavite, Laguna, Quezon, Batangas and Pangasinan. Samples of flowers in the vicinity of the apiaries were collected and examined in the laboratory for the presence of mites.

RESULTS AND DISCUSSION

The duration of the different stages of *V. jacobsoni* was not determined in the laboratory. All the adults died after 72 hours. The nymphs survived only for 12 hours despite the frequent supply of fresh pupae. The mites introduced to the adult bees likewise died before egg laying. Increased nymphal mortality may be due to the differences in temperature in the hive and laboratory. The hive temperature (34-35°C) is higher than laboratory temperature (27°C). Nutrition may also be a factor. In excised pupae, the quality of the haemolymph may be reduced compared to when they were intact and undisturbed in the brood cells. Because of the constraints in the laboratory, observations were done mainly from the standing colony.

The life cycle of *V. jacobsoni* occurred primarily inside the bee cell. They developed and multiplied in the capped brood cell. The female mite entered the cell of the late larval instar of bee prior to capping. When the cells were uncapped and individually examined, the eggs (Fig. 1), protonymph (Fig. 2), deutonymph (Fig. 3) and adult (Fig. 4) were present. The eggs were laid singly at the bottom and wall of the bee cell. Sometimes, the eggs were attached to the bee larvae. The mite completed its development inside the bee cell and the female mites attached themselves to the emerging adult bees while the male stays inside the bee cell.

Another sign of mite infestation was the presence of white spots at the bottom of the bee cell. Akratanakul (1975) believed these to be feces of mites.

Toxicity Studies

By analysis of variance, significant differences were obtained from the different treatments. Highest mite mortalities were obtained from naphthalene and Tetradifon-treated colonies (Table 1). Among the plant materials tested, *H. suaveolens* and *G. sepium* were the most effective followed by *P. odorata.* No mortality was observed in *B. balsamifera* and control. This indicates that naphthalene may be substituted for the more expensive Tetradifon strips. However, treatments must be done before honey flow to avoid possible honey contamination. Three treatments were proven sufficient to suppress mite population.

If the utilization of plant materials will be explored, *H. suaveolens* and *G. sepium* seem promising.

Distribution of V. jacobsoni

V. jacobsoni was present in all apiaries visited throughout the year. Peak mite populations were from October to May. The cosmopolitan distribution of bee mites suggests that they could thrive on a wide environmental gradient.

No bee mites were found in all flowers collected from the vicinity of the bee farms surveyed. This supports the fact that *V. jacobsoni* is a true obligate parasite of bee, hence it can not survive without its host (Shabanov *et al,* 1978).

SUMMARY AND CONCLUSION

1. The life cycle of bee mite, *Varroa jacobsoni* occurs primarily in the bee cell. The female mites attach themselves to the emerging adult bees while the males stay inside the cell.

2. High mortalities were observed from naphthalene and Tetradifon–treated colonies and those treated with *H. suaveolens* and *G. sepium.*

ACKNOWLEDGEMENT

The authors wish to acknowledge Mr. Angel Velasco for allowing the use of his bee farm in Sariaya, Quezon for the toxicity studies.

REFERENCE

1. Akratanakul, P. 1975. Biology and Systematics of Bee Mites of the family Varroidae (Acarine: Mesostigmata). M.S. Thesis, Oregon State University. 64 pp.
2. Choi, S.Y., and K.S. Woo. 1973. Studies on the bionomics of bee mite, *Varroa jacobsoni* Oudemans and its chemical control. Research Reports of the Office of Rural Development. Suwon, Korea 15 (Livestock 35-43).
3. Coleman. 1962. Acarine Strike. *Gleamings of Bee Culture.* **90**(2):87.
4. De Jong, D., R.A. Morse and G.C. Eickwort. 1982. Mite pests of honeybees. *Ann. Rev. Ent.* **27**: 229-252.
5. Delfinado, M.D. 1963. Mites of honeybees in Southeast Asia. *J. Apic. Res.* **2**: 113-114.
6. Laigo, F.M., and R.A. Morse. 1968. Control of bee mite *Varroa jacobsoni* Oudemans and *Tropilaclaps clarae* Delfinado and Baker with chlorobenzilate. *Philipp. Ent.,* 144-148 p.
7. Pandey, R.S. 1967. *Varroa jacobsoni:* A new mite infesting honeybee *(Apis mellifera)* colonies in India. *Bee World.* **48**:16.
8. Renninghoff, V., and W. Kitter. 1984. Field trials to control the bee mite, *Varroa jacobsoni* with Folbex R-VA and biotechnical measures on bee farm in Tunesia. *Anim. Res. and Dev.* **19**: 94-103.
9. Sakai, T., K. Takeuchi and A. Hara. 1979. Studies on the life history of a honeybee mite, *Varroa jacobsoni* Oudemans in laboratory rearing. Tamagawa Univ. *Bull. Fac. Agric.* **19**: 95-123 (In Biol. Abst. **70**(8): 51293).
10. Sevilla, V.J. 1963. Observations of the life history and habits of three new acarine pests of honeybees in the Philippines. Undergraduate Thesis. Univ. of the Philippines, Los Baños, 42 pp.
11. Shabanov, M., S. Nedealkov and A. Toshkov. 1978. Varroatosis – a dangerous parasitic disease of bees. *Am. Bee J.* **118**: 402-403.

Table 1. Mean percent mortality of *V. jacobsoni* at various treatments.

Treatments	Mean Percent Mortality	
Tetradifon	65.13	ab
Naphthalene	71.57	a
Hyptis suaveolens	33.33	b
Premna odorata	6.67	c
Gliricidia sepium	32.67	b
Blumea balsamifera	0	d
Untreated	0	d

Means with the same letter(s) are not significantly different using DMRT.

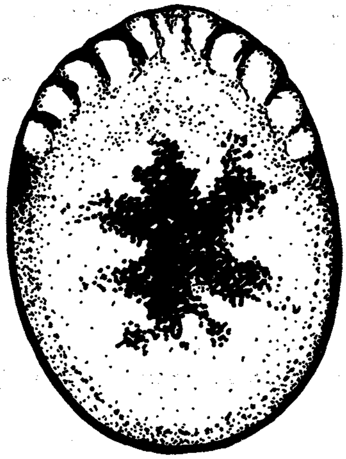

Fig. 1. An egg of Varroa jacobsoni *(250 X)*

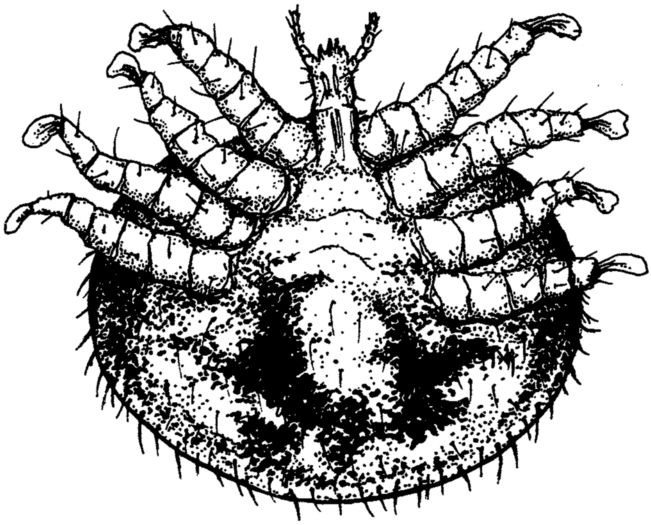

Fig. 2. Line drawing of protonymph of V. jacobsoni *(ventral view. 250 X)*

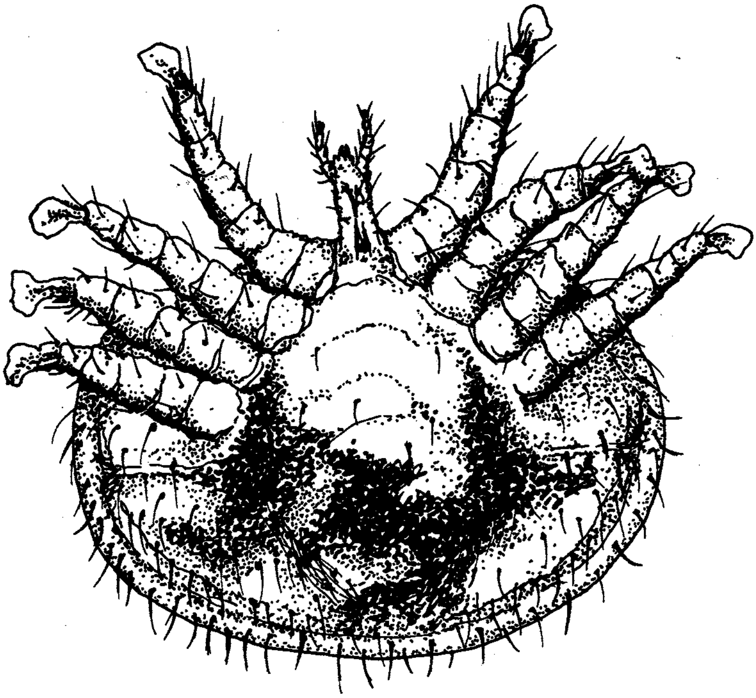

Fig. 3. Line drawing of deutonymph of V. jacobsoni *Oudemans (ventral view, 250 X)*

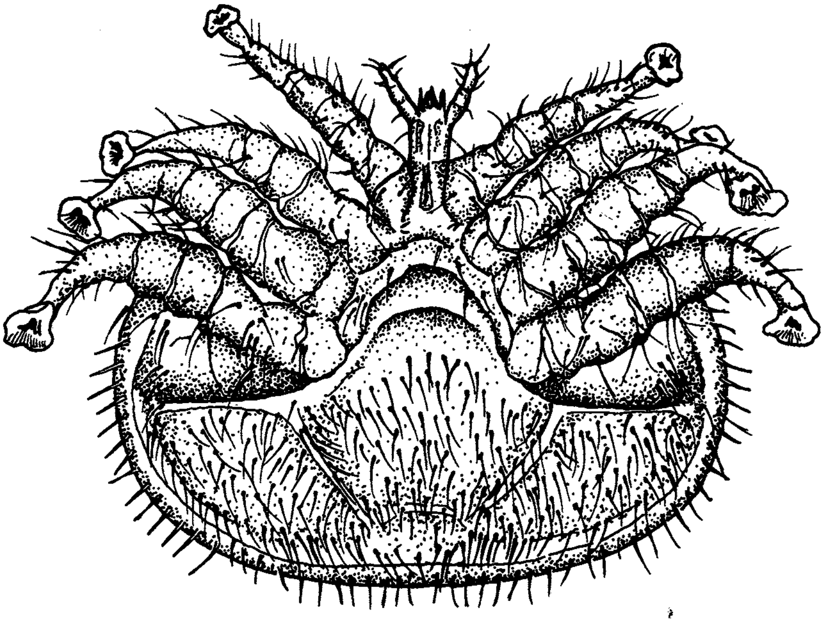

Fig. 4. Line drawing of adult female of V. jacobsoni *(ventral view, 250 X)*

TENSILE STRENGTH AND REFRACTIVE INDEX OF POLYACRYLONITRILE-METHYLACRYLATE (PAN-MA) COPOLYMER FILMS

N. GOGULAMURALI and D. RAMASWAMY
Department of Physics
Presidency College
Madras 600 005, India.

ABSTRACT

The tensile strength and refractive index of polyacrylonitrile-methylacrylate (PAN-MA) in the ratio of 94%:6% (System I) and 97%:3% (System II) are investigated and the contribution of methylacrylate (MA) content to the stiffness and clarity of the copolymer film, (PAN-MA) is interpreted. From the values obtained, the increase in MA content decreases the stiffness and clarity of the film. The morphological studies of the control and fractured surfaces also reveal that higher MA content in the Copolymer PAN-MA decreases the fibrillar nature and in turn the work done to fracture. Therefore System II appears to be more compatible with that of leather as surface coating material than System I.

INTRODUCTION

The study of tensile behavior and physical properties of acrylic copolymers in the form of films is important since it finds a variety of applications as a structural material in many fields. Among the copolymers, polyacryolonitrile (PAN) usually forms the main constituent. Though this polymer is mainly used for synthetic fibers, the film form of a copolymer with PAN finds application as a coating material for leather with glossy surface. This aspect is important from the commercial point of view. In order to make such a choice possible, the mechanical properties of polymers in general are often assessed (Susan, 1984; Buskina, 1981; Uchastkin, 1980) and in particular acrylics have been extensively investigated in this regard by many investigators (Rajadurai, 1982; Allyn, 1967).

In this particular study, the tensile properties and refractive index of PAN cross-linked with 6% and 3% of MA were studied to find out the compatibility of this particular polymer with that of leather. The morphology of the control and fractured surfaces were also studied using Scanning Electron Microscopy (SEM).

MATERIALS AND METHODS

Two copolymers, *viz.,* PAN-MA in the ratio of 94%:6% (System I) and 97%:3% (System II) were used. Films of these copolymers were cast from the solution.

PAN-MA copolymers were dissolved in dimethyl formamide (DMF) (Bikales, 1971). These solutions were then spread over a well cleaned, smooth-surfaced glass plate and dried. Then the films were separated from the glass plate and conditioned at 25 ± 2°C and 75 ± 2% r.h.

Determination of Tensile Strength:

The mechanical properties of the copolymers (Systems I and II) were determined, using an Instron Universal tensile testing machine (model 1112). Using a die, the specimens of the films were cut out according to ASTM procedure (ASTM, 1965). Test samples of even thickness were selected using a thickness gauge with a sensitivity of the order of 0.0001 in. Films were suitably mounted on a cardboard and conditioned again as described above. Individual test films were removed from the desiccator and immediately tested. The tensile strength and percentage of elongation were calculated as follows:

$$\text{Tensile strength} = \frac{\text{Breaking load (kg)}}{\text{Cross-sectional area (mm}^2\text{)}}$$

$$\text{Percentage of elongation} = \frac{\text{Increase in length}}{\text{Original length}} \times 100$$

$$= \frac{\text{length in chart/magnification ratio}}{\text{Original length}} \times 100$$

$$\text{Where Magnification ratio} = \frac{\text{Chart speed}}{\text{Cross head speed}}$$

Refractive Index Determination:

The refractive indices of Systems I and II were determined using Michelson's interferometer, using the formula,

$$2(\mu - 1)\ t = N\lambda.$$

Where N – the shift in fringes
λ – wavelength of the light used
t – thickness of the polymer film
μ – the refractive index of the film.

Scanning Electron Microscopy (SEM):

The specimens were cut and stuck to aluminum stubs with a colloidal suspension of silver and coated all over to a thickness of about 20 nm with gold using Edwards Sputter Coater unit. This ensures that the samples were electrically conducting. The samples were then introduced into the specimen chamber of a Cambridge Stereoscan S 150 SEM and scanned with a filament current of 2.5Å at an operating voltage of 8 KV. Scanning electron micrographs were obtained for the intact films as well as for the films on which mechanical testing had been carried out.

RESULTS AND DISCUSSION

The calculated values of the tensile strength, typical modulus, work done to fracture and refractive index of the copolymeric films are shown in Table 1. In view of the higher boiling point of DMF, generally the films had to be cast at 70°C. Since Flory's type analysis (Strepikheyev, 1971; Tager, 1972) would predict a glass transition temperature T_g = 100°C for Systems I and II, the casting of the films at 70°C was not expected to alter the properties of the films.

Stress-strain behavior of Systems I and II are shown in Figs. 1 and 2 respectively. The two copolymers derived from Systems I and II show lesser extension at break but higher strength. Upon comparing Systems I and II, the stiffness of the film increased by increasing the amount of PAN content. Therefore MA contributes to the stiffness of the film as shown by the modulus values and the clarity of the film also increased by increasing PAN content.

The surface structure of cross sections of the films are shown in Figs. 3 and 4 for Systems I and II. The cross-sections of the Systems I and II copolymers have pores more randomly distributed. The fracture surfaces of the materials observed in the SEM are shown in Figs. 5 and 6. The fracture surfaces show fibrous components. In the copolymers with more PAN (97%) more fibrils are seen. The more the fibrillar pull out, the higher the work done to fracture the sample as shown in Table 1. For polymers tested below T_g (present case), the fibrillar structure increases the work done to fracture.

CONCLUSIONS

1. PAN contributes to packing density or fibrillar content and MA contributes to the stiffness and the clarity of the PAN-MA copolymer film.

2. Of the two systems, System II with good flexibility, hardness and clarity (which is necessary for a surface coating material over leather) was found to be a suitable blend for a surface coating material.

ACKNOWLEDGEMENT

We are grateful to the Professor and Head of the Department, Dr. T.K. Palanisamy, for permitting us to publish this paper. We also thank him for his suggestions during the course of this work.

REFERENCES

1. Allyn, G. 1967. Film forming Composition, Myers and Long ed., 1st. ed., Marcel Dekker, Inc., Newyork V.1.
2. Bikales, Norbert M. 1971. Mechanical Properties of Polymers. 1st ed., Wiley – Interscience, New York.
3. Buskina, L.G., B.E. Geller, I.Z. Zakirov, and A. Ulmasov. 1981. Preparation of a fibre from products of the graft copolymerization of acrylonitrile, methylacrylate and natural wool wastes. (USSR) *Khim. Volokna.* **1**: 39-40.
4. Methods of Tensile Testing D-1708. 1965. Book of ASTM Standards, Part 27, American Society of Testing and Materials, Philadelphia.
5. Rajadurai, S., and S. Kulasekharan. 1982. Acrylics and their uses in leather manufacture. 1st. ed., NICLAI, C.L.R.I. Madras-20, India.
6. Strepikheyev, H., V. Derevtskaya and G. Slonimsky. 1971. A First Course in Polymer Chemistry, 2nd. ed., MIR Publishers, Moscow.
7. Susan, J., and M.S. Spring. 1984. The effects of binder film characteristics on granule and tableting properties. *J. Pharm Pharmacol.* **36**(7): 421-26.
8. Tager, A. 1972. Physical Chemistry of Polymers, 1st. ed., MIR Publishers, Moscow.
9. Uchastkin, V.I., and N.A. Yakovlev. 1980. Relations between the parameters of elasticity and stress causing failure of certain polymers. (USSR). *Plast. Massy* 7:57-58.

Table 1. Tensile properties and refractive index of Systems I and II.

S. No.	Sample	Thickness of the film (mm)	Breaking Strength (kg/mm^2)	Breaking Strain	Typical modulus	Work to fracture (J/m^3)	Refractive index
1.	PAN-MA (94%-6%) (System I)	0.045	3.96 ± 0.20	8.17%	0.5	1.87 x 10^2	1.51
2.	PAN-MA (97%-3%) (System II)	0.067	2.31 ± 0.71	23.08%	0.1	3.15 x 10^2	1.59

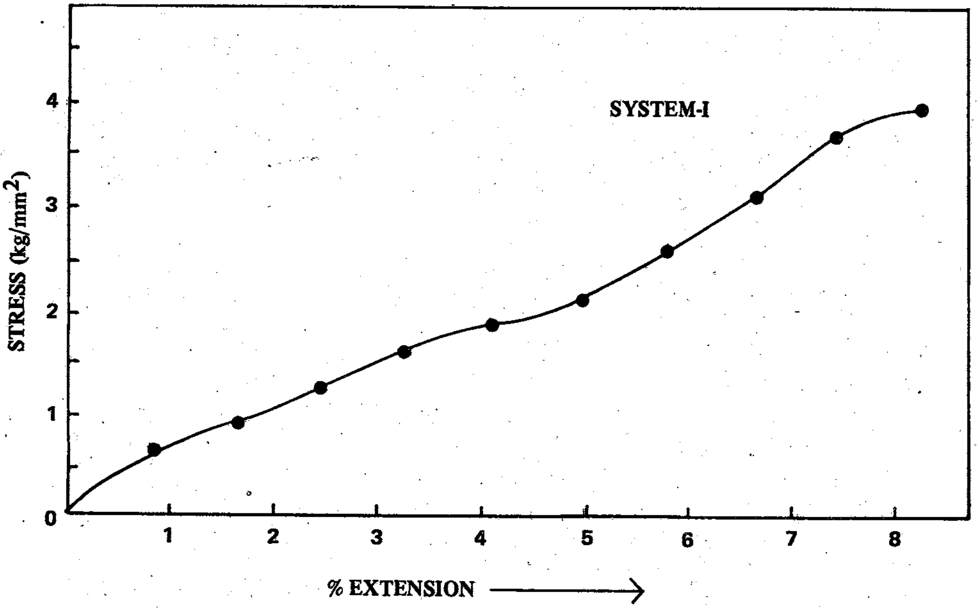

Fig. 1. Stress-strain behavior of System I.

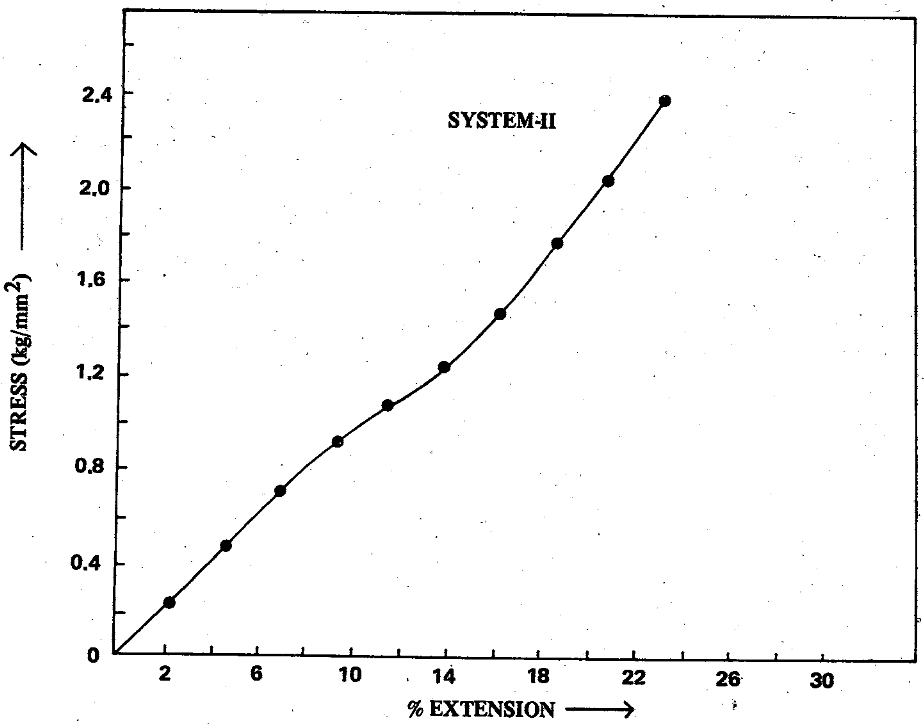

Fig. 2. Stress-strain behavior of System II.

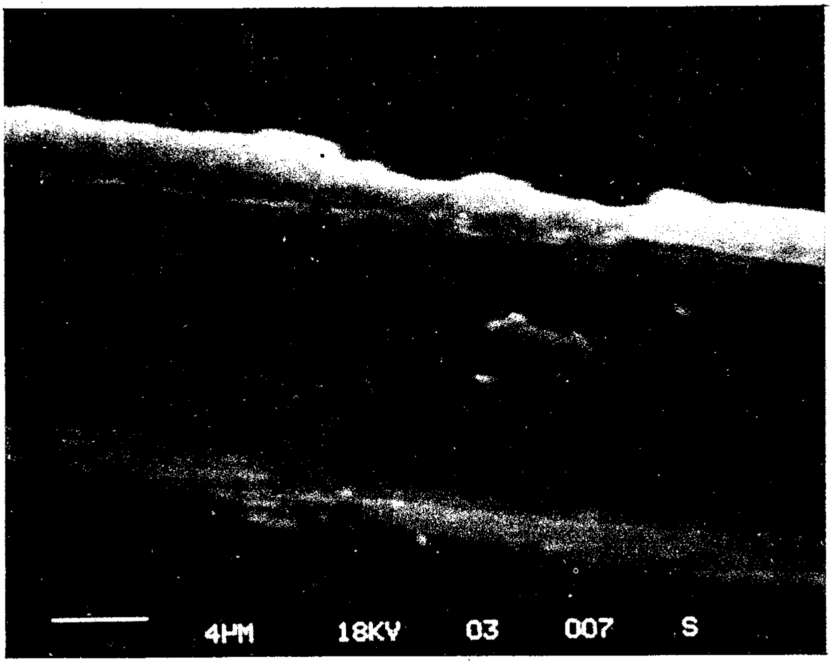

Fig. 3 Surface structure of cross-section of System I

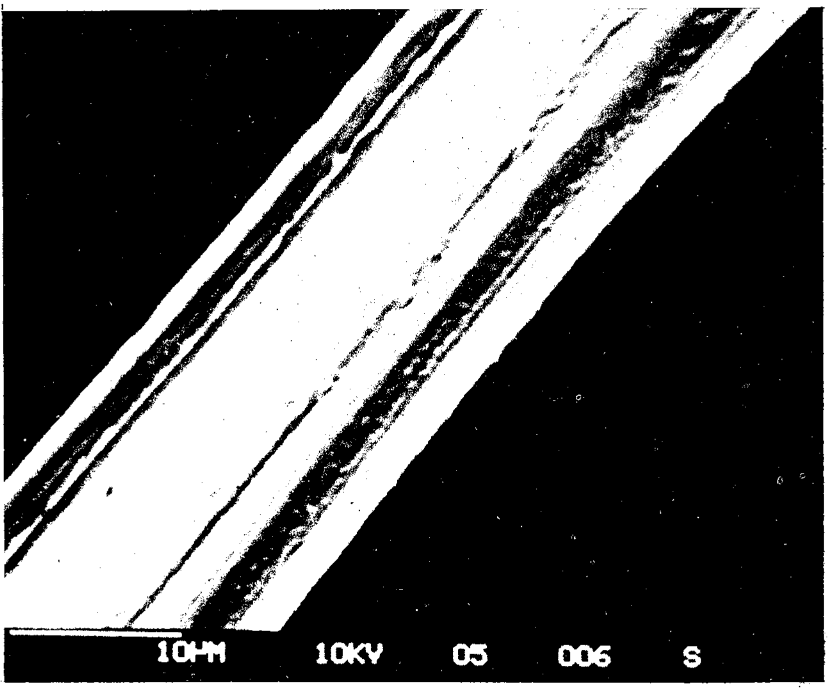

Fig.4 Surface structure of cross-section of System II

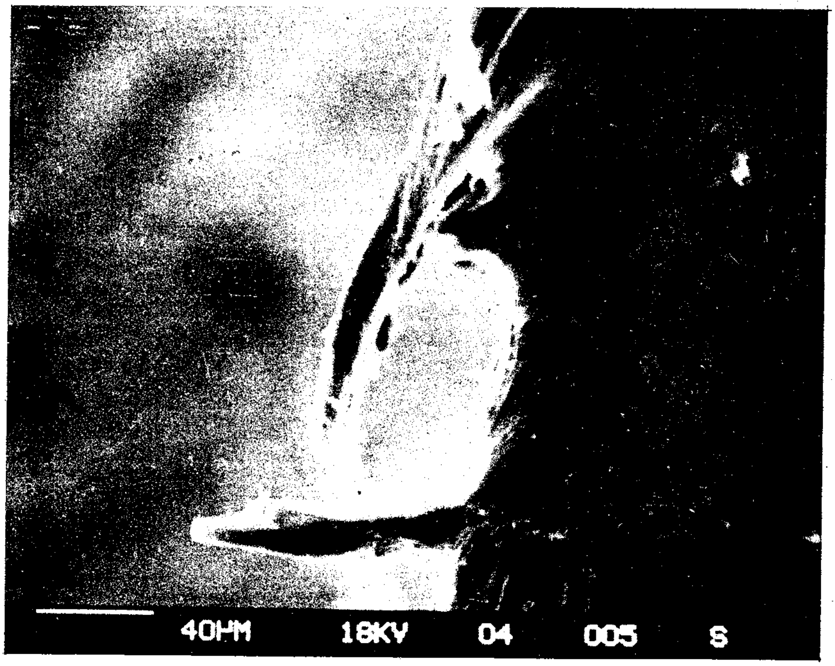

Fig. 5. Fractured surface of System I. (Lower PAN content).

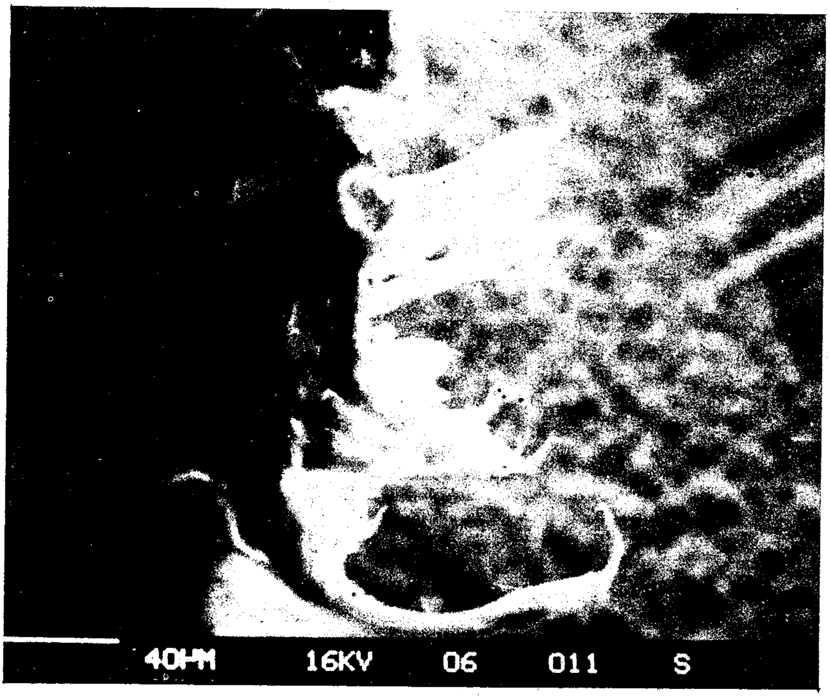

Fig. 6. Fractured surface of System II. (Higher PAN content).

TENSILE STRENGTH AND ELASTICITY OF 2 ETHYL HEXYL ACRYLATE-METHYLMETHA ACRYLATE-ACRYLONITRILE (2 EHA-MMA-AN) COPOLYMER FILM

N. GOGULAMURALI and D. RAMASWAMY
Department of Physics
Presidency College
Madras 600 005, India

ABSTRACT

The tensile strength and elasticity of copolymer 2 ethyl hexyl acrylate-methylmetha acrylate-acrylonitrile (2 EHA-MMA-AN) – based soft resin was studied using Instron tensile testing machine. It has been found to exhibit plastic flow at room temperature, high extensibility, low tensile strength and more work to fracture. The distribution of micro and macro voids, fibril formation and other properties that affect the tensile strength and elasticity of this polymer 2 EHA-derived resin were also investigated.

INTRODUCTION

It is now known that weather conditions have a large influence on the usability of acrylate films in surface coating applications (Encycl. Poly. Sci. and Tech., 1970; Rajadurai, 1982). For example, a material like leather, when finished with an acrylic resin and subjected to cold climatic conditions, the cold cracking resistance of the film becomes important (Rajadurai, 1982). In this regard, monomers like 2 ethyl hexyl acrylate is of major importance because they yield polymers with very low glass transition temperature, T_g, viz., 30°C to 60°C. There are now several commercial formulations of acrylic resins derived from 2 ethyl hexyl acrylate copolymers for application as surface coating materials suited for leather, textiles, etc. Generally, in the choice of polymers for use in films for specific purposes, the two important criteria used are tensile strength and elasticity (Neilsen, 1965; Encycl. Poly. Sci., 1968). The examination of the surface of the polymer film is made by Scanning Electron Microscopy (SEM) since the presence of voids, inclusions and defects are known to affect the mechanical properties of a polymeric film (Bikales, 1971).

Since 2 ethyl hexyl acrylate is often used as a co-monomer in the preparation of cold crack resistant resins, it seems worthwhile to evaluate the mechanical properties like tensile strength and elasticity of such resins at least at room temperature.

MATERIALS AND METHODS

2 ethyl hexyl acrylate-methylmetha acrylate-acrylonitrile (2 EHA-MMA-AN)-based soft resin was obtained in the form of a white emulsion. It was spread over the smooth surface of a cleaned glass plate. The solvent was allowed to evaporate at room temperature. Then the film was removed from the glass plate and kept in a thermostat (60°C) for

ten minutes to remove moisture in the film. When it was completely dried and the sticky nature was removed, the film was preserved in between two filters papers. The tensile strength and elasticity of copolymer 2 EHA-MMA-AN film was studied using Instron tensile testing machine (model 1112) and the scanning electron micrographs were obtained both for the intact film and the film on which mechanical testing had been carried out using a Cambridge Stereoscan S150 Scanning Electron Microscope. The uniformity of distribution of micro and macro voids, fibril formation, and other properties were investigated by scanning electron microscopy. The load-elongation graph was obtained from the chart attached to a recorder of the Instron.

RESULTS AND DISCUSSION

The stress-strain behavior of the 2 EHA-MMA-AN film is shown in Fig. 1. The 2 EHA terpolymers show very high extension at break and low tensile strength. The modulus at any extension is also low indicating that it forms a very flexible film. The work to fracture is high for the material which is again a reflection of the considerable extension the material undergoes before fracture. The modulus values are given at three different extensions due to different behavior of the film at these extensions. Above an extension of 200%, the sample shows a flow region where extension is accompanied by little increase in load. This is seen up to an extension of 400% after which the load taken up is comparatively rapid. This is typical of samples which are tested above the glass transition temperature T_g, since T_g of this polymer, using Flory's type analysis, has been calculated to be 20°C. The observed stress behavior is as expected. The surface section structure of cross-section and the fractured surface of the film observed in SEM is shown in Figs. 2 and 3. It has sets of pores distributed at different places in the film. These voids can act as weak points in initiating fracture. The fractured surface shows plastic flow. The amorphous nature of the material is also seen.

Thus it is obvious that polymers which are tested above T_g exhibit plastic flow at higher extensions and higher work done to fracture.

The polymer 2 EHA-derived resin investigated has T_g which is far lower than room temperature. It therefore exhibits plastic flow at room temperature, high extensibility, low tensile strength and more work to fracture. The values are given in Table 1.

REFERENCES

1. Bikales, Norbert M. 1971. Mechanical Properties of Polymers. 1st ed. Wiley-Interscience, New York.
2. Encyclopedia of Polymer Science and Technology. 1969. Interscience, New York V.8.
3. Encyclopedia of Polymer Science and Technology. 1970. Interscience, New York V.18.
4. Neilsen, L.E., and Reinhold. 1965. Mechanical Properties of Polymers, 3rd ed., New York. V.1.
5. Rajadurai S., and S. Kulasekharan. 1982. Acrylics and their Uses in Leather Manufacture. 1st. ed., NICLAI, C.L.R.I., Madras-20, India.

Table 1. Tensile properties of 2 EHA-MMA-AN copolymer film.

	Thickness of film (mm)	Breaking strength (kg/mm^2)	Breaking strain (%)	Typical modulus	Work to fracture (J/m^3)
2 EHA-MMA-AN	0.260	0.250 ± 0.038	754.17	4.41×10^{-4} 1.67×10^{-4} 6.81×10^{-4}	9.78×10^2

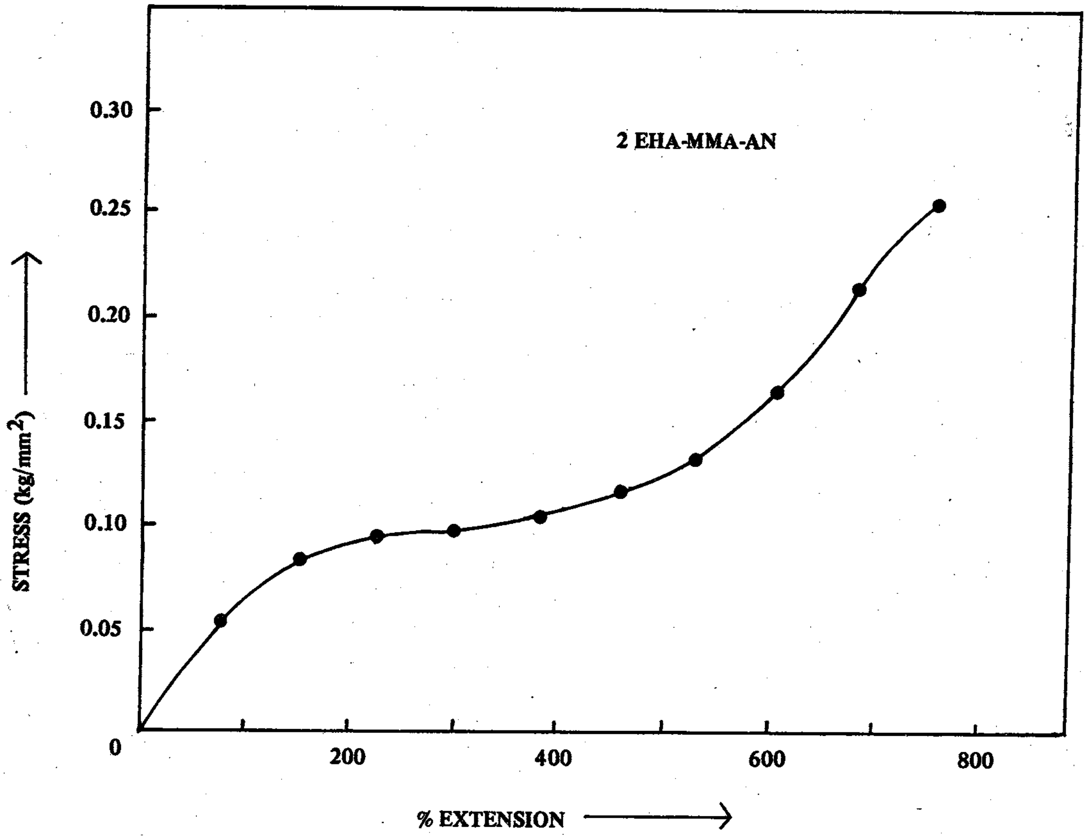

Fig. 1. Stress-strain behavior of 2 EHA-MMA-AN copolymer.

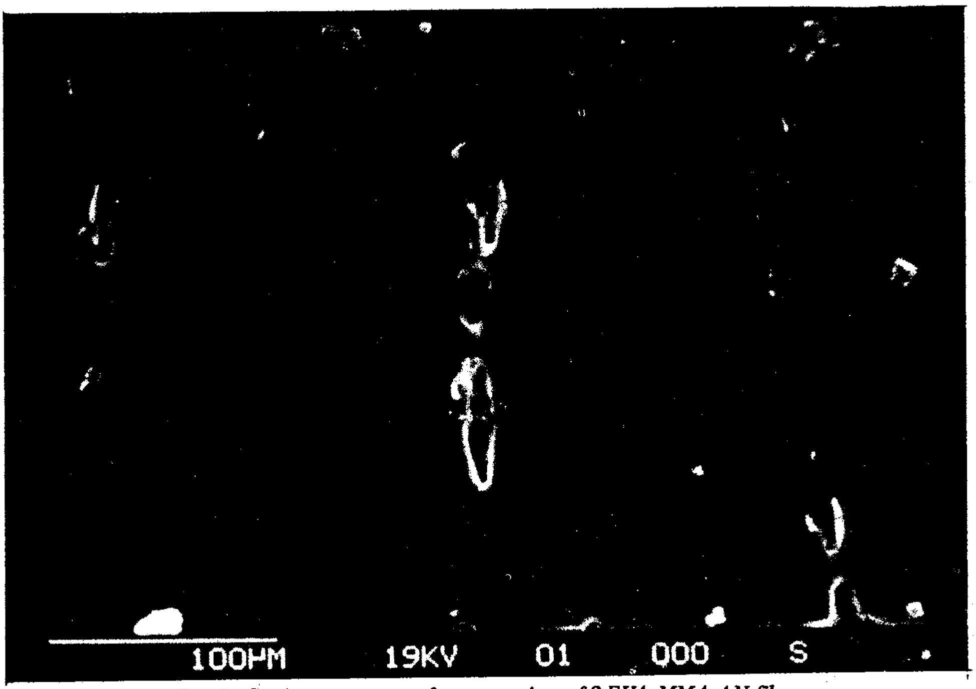

Fig. 2. Surface structure of cross-section of 2 EHA -MMA-AN film.

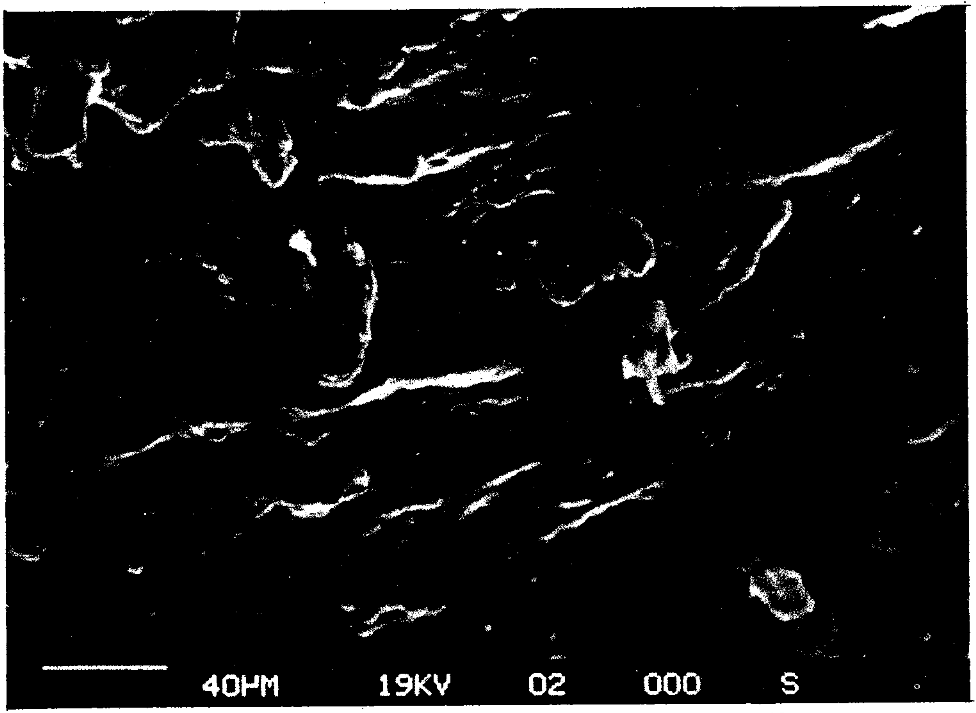

Fig. 3. Surface structure of fractured surface of 2 EHA-MMA-AN film.

GUIDE TO AUTHORS

1. Manuscripts intended for publication in the Philippine Journal of Science should be sent to the Editor, Philippine Journal of Science, National Institute of Science and Technology, P.O. Box 774, Manila, Philippines.
2. The Journal will not be responsible for the loss of unsolicited manuscripts, but those received will be acknowledged and considered promptly by the Board of Editors. Authors will be notified of the decision reached.
3. Manuscripts on biology must be accompanied by abstract for publication in the biological abstract.
4. Manuscripts submitted should be typed on one side of white bond paper, 8½" X 11", and double-spaced throughout. One original copy and one carbon copy of manuscripts should be submitted.
5. Illustrations (use tracing paper) should accompany manuscripts on separate sheets. Photographs should be sent unmounted, with serial number written on back to correspond with list of captions.
6. References are indicated by the author's surname and year in parenthesis in the text.

   Example: The rich flora of the Phil. numbering some 10,000 or more species (Quisumbing, 1951) provide an almost inexhaustible source of materials for study.

7. Manuscript submitted should consist of the following parts in this order:
   a. Title of the article (all capital letters)
   b. Name and address of author
   c. Abstract – to contain a brief indication of what was done and the significant results and conclusions for the general readership.
   d. Introduction
   e. Materials and Methods
   f. Results and Discussion
   g. Summary/Conclusions/Recommendations (as needed) – to contain an enumeration of the major findings/conclusions/recommendations.
   h. Acknowledgement (if any)
   i. References

8. Please take note of the following styles for reporting references: – Citation of a journal article:
   1. Author's name
   2. Year
   3. Title of the article
   4. Full name of the Journal (abbreviated)
   5. Volume and number
   6. Pages

   Examples: Velasquez, G.T. 1979. The microscopic algae in the hard coral communities. *Philipp. J. Sci.* 108 (3-4): 121-135.

– Citation of a book

1. Author's name
2. Year of publication
3. Full title of the book
4. Number of edition
5. Name and place of publisher
6. Volume

Example: Smith, J. 1957. Textbook of Chemistry, 3rd ed., Elsevier, Amsterdam, V.*2*.

– Citation of a patent:

1. Inventor's name
2. year
3. kind and number of patent
4. year of patent application
5. abstract journal where abstract of the patent can be found

Example: Smith, J. 1961. U.S. Patent 2 542 356 (1952) Chem. Abstr. *51*, 2670.

– Citation of Thesis:

1. name of author
2. year
3. Kind and title of thesis
4. place where thesis was done
5. address

Example: Kintanar, Q. 1969. Studies on the mechanism on the fatty liver and the hypolipidemia induced by orotic acid in the rat. Ph. D. thesis. John Hopkins University, Baltimore Maryland, U.S.A.

9. Please arrange references alphabetically.

10. Please use the metric system in reporting such as:

Length

meter	m
millimeter	mm
centimeter	cm

Volume

liter	L
milliliter	mL
cubic meter	m^3
Energy and Work	KJ

kilojoule (replace calorie in dietetics)

Mass

kilogram	kg
gram	g
ton (metric ton)	t
milligram	mg

Time (same units used in both Metric and English System)

day	d
hour	h
minute	min
second	s

Amount of substance

mode	mole

Temperature

degree celsius	°C

PUBLICATIONS AVAILABLE

CHECKLIST OF THE ANTS (HYMENOPTERA: FORMICIDAE) OF ASIA. By J. W. Chapman and S. R. Capco. Institute of Science and Technology Monograph 1 (1951) new series. Paper, 372 pages. Price, US$2.00.

NOTES ON PHILIPPINE MOSQUITOES, XVI, GENUS TRIPTEROIDES. By F. E. Baisas and Adela Ubaldo-Pagayon. Institute of Science and Technology Monograph 2 (1952) new series. Paper, 198 pages with 23 plates and four text figures. Price US$2.50.

A REVISION OF THE INDO-MALAYAN FRESH-WATER FISH GENUS RASBORA. By Martin R. Brittan, Institute of Science and Technology Monograph 3 (1953) new series. Paper, 224 pages with three plates and 52 text figures. Price, US$ 2.50.

SECURING AQUATIC PRODUCTS IN SIATON MUNICIPALITY, NEGROS ORIENTAL PROVINCE, PHILIPPINES. By Donn V. Hart. Institute of Science and Technology Monograph 4 (1956) new series. Paper, 84 pages with 22 text figures and eight plates. Price, US$1.25.

AN ECOLOGICAL STUDY OF THE KOUPREY, NOVIBUS SAUVELI (URBAIN). By Charles H. Wharton. Institute of Science and Technology Monograph 5 (1957) new series. Paper, 111 pages with 11 plates and 16 text figures. Price, US$1.25.

FERN FLORA OF THE PHILIPPINES. By Edwin B. Copeland. Institute of Science and Technology Monograph 6, Vols. 1-3 (1958-1960) new Vol. 2, 193-376 p., Paper, Price, $1.75; Vol. 3, 377-577 p., Paper, Price, US$1.75.

THE PHILIPPINE PIMPLINI, POEMENIINI, RHYSSINI, AND XORIDINI. By Clare R. Baltazar. National Institute of Science and Technology Monograph 7 (1961) new series. Paper, 120 pages with four plates. Price, US$1.50.

PACIFIC PLANT AREAS. Edited by C.G.G.J. Van Steenis. National Institute of Science and Technology Monograph 8, Vol. 1 (1963) new series. Paper, 246 pages with 26 maps. Price, US$3.00.

INDEX TO THE PHILIPPINE JOURNAL OF SCIENCE, VOL. 1 (1906) TO VOL. 109 (1980). Vol. 1-10 (1906-1915); Vol. 57-79 (1936-1950); Vol. 80-99 (1951-1970); Vol. 100-104 (1971-1975); Vol. 105-109 (1976-1980). Monograph of the National Institute of Science and Technology. Price per bounded copy, US $3.00.

THE ARCHAEOLOGY OF CENTRAL PHILIPPINES. By Wilhelm G. Solheim, II. National Institute of Science and Technology Monograph 10 (1964) new series. Paper, 235 pages with 29 text figures and 50 plates. Price, US$3.00.

THE PHILIPPINE CERAMIC RAW MATERIALS. Special issue of the Philippine Journal of Science for 1984. 226 pages. Price, US $14.00 per copy.

NITROGEN FIXATION. Special issue of the Philippine Journal of Science for 1985. 143 pages. Price, US$6.00.

INFORMATION FOR AUTHORS AND SUBSCRIBERS

The Philippine Journal of Science is a non-profit publication and accepts only original unpublished works from both here and abroad. Manuscripts submitted must conform to our guidelines (see GUIDE TO AUTHORS), and will be published only after passing the critic's evaluation and after the author/s have complied with all the requirements for its publication.

Annual subscription rates are: Local, ₱120.00 and Foreign, US $22.00 inclusive of postage. Beginning 1986, individual copies of any of the quarterly issues maybe purchased at ₱45.00 per copy for issues containing one number or ₱60.00 for issues containing two numbers inclusive of postage. For foreign buyers, the corresponding cost per copy in US dollars are: US $8.00 and US $11.00 respectively. Reprints of articles are available at rates of ₱0.50 for local and US $0.50 for foreign buyers per page inclusive of postage.

Please make checks payable to: *The National Institute of Science and Technology,* P.O. Box 774 Manila, Philippines.

All communications should be addressed to:

The Philippine Journal of Science
National Institute of Science and Technology
P.O. Box 774 Manila, Philippines.